COCAINE HYPOPHAGIA AND HYPERLOCOMOTION IN RATS BEFORE AND AFTER EXPOSURE TO A HIGH-FAT DIET

A Thesis

by

DAO HONG HO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2003

Major Subject: Psychology

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Approved as to style and content by:

Paul J. Wellman (Chair of Committee)	Jack R. Nation (Member)
Susan E. Maier (Member)	William S. Rholes (Head of Department)

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ABSTRACT

Cocaine Hypophagia and Hyperlocomotion in Rats Before and After Exposure to a High-Fat Diet. (December 2003)

Dao Hong Ho, B.A., University of North Texas

Chair of Advisory Committee: Dr. Paul J. Wellman

Relatively few studies have examined the effects of psychostimulants in obese subjects. Using the dietary obese rat model, the present experiments determined the reductions in food intake (hypophagia) and increases in locomotion (hyperlocomotion) induced by cocaine in diet-induced obese prone (DIO-prone) rats and diet resistant prone (DR-prone) rats as well as diet-induced obese (DIO) rats and diet resistant (DR) rats. In Experiment 1, thirty-six male Sprague-Dawley rats were given intra-peritoneal (i.p.) injections of cocaine (0, 10, 20, and 30 mg/kg) immediately prior to placement into locomotor chambers outfitted with a food source and a water source for a 60-minute test period. In Experiment 2, the same rats were exposed to a high-fat diet, and were subsequently divided into groups according to the extent of the weight gain (high weight gainers – DIO group, low weight gainers – DR group, and residual weight gainers – MIX group). The rats were retested for reactivity to cocaine using conditions similar to those in Experiment 1.

Rats injected with cocaine prior to high-fat exposure (Experiment 1) showed a dose dependent suppression of food intake, as well as a dose dependent increase in locomotor activity, with DR-prone rats exhibiting an enhanced degree of cocaine-induced hypophagia, as well as cocaine-induced hyperlocomotion as compared to the other groups. In Experiment 2, DIO rats exhibited a suppression of food intake after injection of 10 mg/kg cocaine, as well as an increase in locomotor activity that was significantly greater than noted in the other groups. When the results of Experiment 1 were analyzed as a function of prospective body weight gain (as opposed to placement into distinct groups), reactivity to cocaine decreased as body weight gain increased. In contrast, after high-fat exposure and weight gain, increased body weight gain was associated with an increased magnitude of suppression in food intake after cocaine administration. Similar patterns of differential cocaine sensitivity were observed for cocaine hyperlocomotion in Experiment 2. These studies indicate that although the propensity to develop obesity is associated with a diminished cocaine response, cocaine reactivity is enhanced after the induction of obesity.

ACKNOWLEDGEMENTS

I would like to thank Paul Wellman, Jack Nation, Susan Maier, Heather Crawford, Rodrigo Valles, and Robin Johnson for their help in completing this thesis.

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INTRODUCTION

Each year the number of obese individuals increases dramatically in the United States, thus making obesity-related illness the second leading cause of death (Mokdad et al., 2001), and trimming the average adult lifespan by 10 to 20 years (Bor, 2003). Increasing prevalence of obesity has been attributed to the increasing demand or consumption of inexpensive high calorie, high-fat products in combination with an individual's genetic predisposition for weight gain (Levin, 2000). Developing effective treatments requires a better understanding of the causes of obesity. Numerous studies have examined the genetic as well as biological and neurochemical components of obesity by employing obesity mutation models such as the Zucker fa/fa rat, and the obese ob/ob mouse, which are obese due to mutated leptin receptors and defective leptin signaling, respectively (Beck, 2001). In addition to genetic alterations, some research studies have induced obesity by surgically removing neurons containing neurotransmitters and neuromodulators that are involved in the control of food intake. For example, ablation of areas involved in food intake control such as the ventromedial hypothalamus (VMH) produces weight gain that eventually leads to obesity (Schwartz et al., 2000). Also, the removal of estrogen via ovarectomy in female rats, for example, induces weight gain (Geary, 2000).

An animal model that more closely mimics the development of human obesity is one that employs a high energy diet to induce the expression of obesity in rats that are genetically prone to obesity (Levin et al., 1997). Reduced leptin sensitivity, neuropeptide

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This thesis was written in the style and format of European Journal of Pharmacology.

Y (NPY; an orexigenic peptide in brain) dysregulation as well as altered monoamine levels all act to predispose some rats, referred to as diet-induced obese (DIO) rats, to undergo rapid weight gain when exposed to a high-energy diet (30-40% fat). At the beginning of weight gain, DIO rats consume more calories than do diet-resistant (DR) rats that have body weights comparable to chow-fed rats, but "normalize" their caloric intake after long-term exposure to high energy diet, consuming the same amount of calories as DR rats, but maintaining significantly higher body weights (Levin and Sullivan, 1989). This suggests that DIO rats undergo a change in fat metabolism, thermogenisis or body weight set point. Thus, exposure to a high-fat diet reveals both phenotypic as well as genotypic differences between DIO-prone and DR-prone rats that prior to high-fat exposure differed only in genotype.

The social pressure to be thin leads to weight control methods such as exercise and dieting. Moreover, some humans will use psychostimulants such as nicotine, amphetamine as well as cocaine to lose weight (Chen et al., 2001; Wellman et al., 2002; Bellinger et al., 2003). Cocaine dose-dependently reduces food intake (Balapole et al., 1979; Cooper and van der Hoek, 1993; Wellman et al., 2002) and increases locomotor activity in rats (Sell et al., 2000), and acts as an appetite suppressant in humans (Cochrane et al., 1998). Cocaine hypophagia, as well as cocaine hyperlocomotion, has been attributed to the drug's ability to increase synaptic levels of dopamine, norepinephrine, as well as serotonin, via transporter reuptake inhibition (Wellman et al., in press; Wellman et al., 2002; Rothman et al., 2001; Caine and Koob, 1994; Kleven and Woolverton, 1993). Dopamine receptor antagonists attenuate cocaine hyperlocomotion (Schindler and Carmona, 2002), while antagonism of alpha-1 adrenoceptors by prazosin attenuates cocaine's hypophagic (Wellman et al., 2002) as well as cocaine's hyper-locomotor action (Drouin et al., 2002a, Drouin et al. 2002b; Wellman et al. 2002; Berthould et al., 1992).

Although many studies have examined cocaine hypophagia as well as the effects of food deprivation on cocaine action (Stuber et al., 2002; Campbell and Carroll 2001; Cabeza and Carr, 1998), few studies have looked at the effects of obesity on the various actions of cocaine including hypophagia, hyperlocomotion, and reinforcement. Lin et al. (2000) has suggested that obesity (which results in increased plasma leptin levels) due to consumption of high-fat foods can down-regulate leptin receptors in the arcuate nucleus of mice. Given that leptin inhibits both dopamine (DA) and norepinephrine (NE) release via the presence of leptin receptors on DA and NE neurons (Brunetti et al., 1999), alterations in leptin receptor binding between lean and obese animals may have major implications for the capacity of cocaine to suppress food intake and to increase locomotor behavior. Moreover, Pothos (2001) reported that rats that were maintained on a cafeteria diet (resulting in 20 % weight gain) showed increased DA release in the nucleus accumbens measured by microdialysis when given amphetamine compared to normal-fed rats given comparable doses of amphetamine. Paradoxically, in the same study, underfed rats (reduced to 80% of normal body weight) showed a similar increase in DA within the nucleus accumbens. Unfortunately, this study did not assess the impact of either genetic predisposition or the behavioral consequences of increased DA levels in brain in response to amphetamine.

Recent studies have suggested a direct link between drug reinforcement circuits and food control circuits in brain via the existence of leptin receptors as well as NPY receptors in the ventral tegmental area (VTA) and nucleus accumbens (NAcc), both principal sites of cocaine action (Pickel et al., 1998; Figlewicz et al., 2003). Clear evidence of leptin's role in reward efficacy of reinforcing stimuli was seen when intra-ventricular injections of leptin into brain decreased the reinforcing effects of brain stimulation (Fulton et al., 2000). When considered in combination with the evidence that DIO and DR rats differ in leptin sensitivity (Levin and Dunn-Meynell, 2002) as well as NPY regulation (Levin and Dunn-Meynell, 1997), it likely that the sensitivity to cocaine-induced hypophagia and hyperlocomotion will differ between DIO and DR rats. Thus, the primary aim of this study was to examine the role of propensity for obesity, as well as expressed dietary obesity in the modulation of cocaine hypophagia and hyperlocomotion. It was predicted that DIO-prone rats will not differ from DR-prone rats in sensitivity to cocaine action due to minimal differences in the physiological profiles of the groups prior to weight gain. In contrast, after high-fat exposure and weight gain, DIO rats will exhibit heightened sensitivity to cocaine action as compared to DR rats due to physiological differences such as dysregulation of food reward and drug reward systems between the groups. In addition, length of exposure to high-fat diet was predicted to result in increasing sensitivity to cocaine reactivity. The hypothesis was tested by administering multiple ascending doses of cocaine to rats both before and after an 11-week exposure to a high-fat diet and observing changes in food intake and locomotor activity.

A novel aspect of the present research was the concurrent assessment of eating and locomotion in an automated activity chamber. A food source as well as a water source was suspended from the ceiling of each chamber, which allowed for the measurement of changes in eating and drinking after cocaine to be related to the concurrent changes in locomotion produced by cocaine. By analyzing the temporal aspect of food intake (vertical time in the “food zone”), water intake (vertical time in the “water” zone) and locomotor activity (residual zone) simultaneously, it was determined that a decrease in food intake due to cocaine administration was not likely due to a simple change in locomotion that competed or interfered with eating. The results indicated that cocaine sensitivity was less in DIO-prone rats compared to DR-prone and MIX-prone groups, but was greater in DIO rats compared to DR and MIX groups, suggesting that both genetic predisposition to obesity as well as physiological changes that accompany obesity play a role in altering cocaine sensitivity.

EXPERIMENT 1

Materials and Methods

Animals

The studies were approved by the Texas A&M University Laboratory Animal Care Committee. Thirty-six ninety-day old male Sprague Dawley rats (Harlan Industries: Houston, TX) weighing approximately 300-350 grams at the beginning of the study were double-housed briefly in plastic hanging cages and then subsequently single-housed in standard plastic hanging cages for the remainder of the study. Rats were housed in a colony room maintained at 22.0 $\pm$ 1 ºC under a 12hr light/dark cycle (lights on 0300hr) and fed standard chow pellets (Teklad, 8728c) and tap water ad libitum, except when noted below. Rats were tail-marked with a permanent marker for ease of identification.

Drugs

A vehicle saline solution was prepared as 0.9% NaCl in distilled water. Cocaine hydrochloride (kindly donated by Dr. Jack R. Nation) was dissolved in saline vehicle and injected i.p. at a volume of 1 ml/kg. Drug doses (10, 20 and 30 mg/kg) were calculated as the weight of the salt per ml of vehicle solution.

Apparatus

Locomotor activity was recorded using Versamax activity chambers (Model RXYZCM-16) comprised of 16 vertical and 16 horizontal infrared sensors encased in a Plexiglas box (40 X 40 X 30.5 cm) with a hinged lid (43 X 43 cm) with 0.5 cm holes drilled for ventilation. Each of the six chambers was connected to a multiplexor-analyzer (Versamax: Model VMA16) interfaced with an IBM-compatible microcomputer. During the two-hour test session, six standard chow pellets (Teklad, 8728c) held together by a large (5.08 cm) metal binder clip were attached to the top front of the left wall above the infrared beams. A calibrated 100 ml drinking tube (Wahmann) with a metal sipper spout inserted through a hole drilled above the back left corner of the chamber was suspended above the top set of infrared beams. Rats were tested under 40W red light illumination and white noise was generated by a radio. In study 1, each chamber was further divided into four equal chambers via two intersecting Plexiglas sheets, which allowed for the testing of two animals per chamber. Pilot data from this lab showed that this design allowed for a reliable measure of locomotion, food intake, and water intake (Ho et al., 2002). Beam breaks were characterized as either total distance traveled (cm), vertical time (number of seconds spent breaking the upper beams), horizontal time (number of seconds spent breaking the lower beams), and rest time (time spent exhibiting no movements detected by infrared beams).

Procedure

The rats were maintained in the colony room for 4 days prior to the first adaptation day and were handled and weighed one day prior to the first adaptation day. Rats continued to undergo daily handling and weighing. Because only six chambers were available for use in these studies, rats were run in squads of twelve with each squad-test day staggered to allow for a single squad to be run each day. The testing schedule for a single squad is summarized in Figure 1.

A1—I1—I2—A2—I3—I4—**A3**—I5—I6—**D1**—I7—I8—**D2**—I9—I10—**D3**—I11—I12—**D4**

↓	↓	↓	↓	↓
Saline	0 mg/kg cocaine	10 mg/kg cocaine	20 mg/kg cocaine	30 mg/kg cocaine

Fig. 1: Timeline of testing schedule for a single squad for Experiment 1. The letter A indicates an adaptation day. The letter I indicates inter-trial days. The letter D indicates test days. Drug doses were administered in an ascending series, rather than a counterbalanced order, to avoid problems of tolerance/sensitization.

At 1345hr, food and water were removed from the home cage to allow for approximately 1hr food deprivation prior to testing. Rats were weighed at 1430hr and were transported to the testing room containing the locomotor chambers in a plastic container with six drawers (two rats per plastic drawer). At 1445hr, under red light and white noise, the rats were placed in their respective chambers (rats were kept in their respective pairs throughout the study to eliminate novelty) and locomotor activity recording commenced. After fifteen minutes, each rat was injected with the appropriate cocaine dose (0, 10, 20 and 30 mg/kg, i.p.) for the test day (refer to schematic above) or briefly handled (A1 and A2) and placed back in the chamber with food pellet packs and sipper tubes in place. Pellets were weighed to the nearest 0.1g, and calibrated water tubes were read to the nearest ml. After 1hr, the food was removed, weighed and returned, and the water tubes were read and returned. This procedure was repeated at the end of the second hour interval. Food intake, but not water intake, was corrected for spillage after each of the one-hour testing periods. Little spillage for both food and water were noticed in rats run in a similar fashion (Ho et al., 2002). Rats were then transported back to the colony room and again allowed food and water ad libitum in the home cage until the next test day. Testing chambers were thoroughly cleaned with a mild Lysol (1 teaspoon per 10 oz) solution and dried with paper towels after each test session. During the two inter-trial days, positioned between each drug test day, rats remained in their home cages and did not undergo any of the testing procedures.

Data Analyses

Versamap software (Accuscan Instruments; Columbus, OH) allowed for the analysis of locomotor data according to experimenter-defined zones. Vertical activity that occurred in a 5.08 X 5.08 cm zone located in the front left corner of the chamber where the food was located was defined as food related locomotor activity. Similarly, vertical activity that occurred in a 5.08 X 5.08 cm zone located in the back left corner of the chamber where the metal sipper tube spout extended through the top of the chamber was defined as water related locomotor activity. In the “food zone” and “water zone”, only vertical time was examined since only a rearing posture most likely indicated feeding or drinking behaviors. “Control zones” were assigned to areas comparable to “food and water zones” (refer to Figure 2) and vertical activity in all four zones was compared to determine the validity of the measure. The entire floor of the chamber was analyzed in terms of total distance traveled as well as rearing (vertical activity) to determine non-food and non-water related locomotor activity.

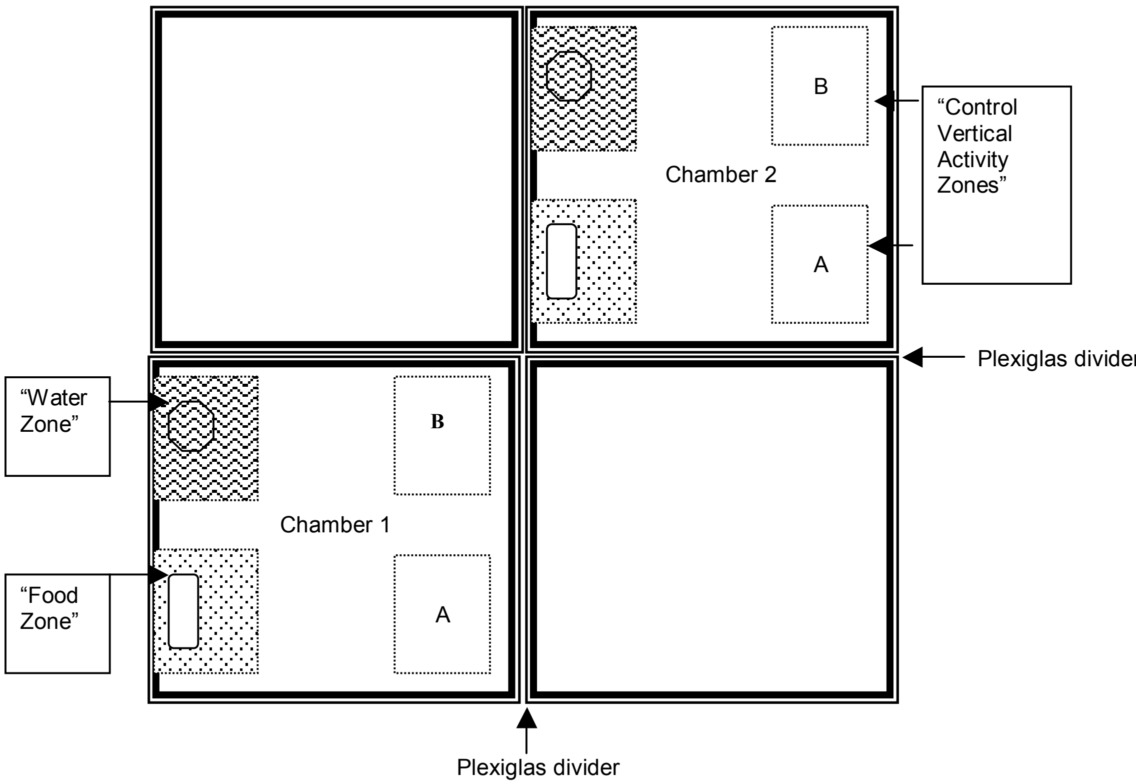


Fig. 2: A schematic of the locomotor activity chamber used to assess food intake and locomotor activity during the test period for Experiment 1. The view is from the top of the chamber looking down onto the chamber floor. Each chamber is 20 X 20 X 30.5 cm with food, water and control zones each measuring 5.08 X 5.08 X 30.5 cm.

Rats, excluding those in the CON group, were divided into groups based on subsequent body weight gain after exposure to the high-fat diet for 4 weeks after the end of Experiment 1. The top nine weight gainers and bottom nine weight gainers were assigned to DIO and DR groups, respectively. The intermediate nine weight gainers were assigned to the MIX group. The overall design of this study was a between group factor of prospective groups (determined at the end of Experiment 1 and prior to Experiment 2) and within group factor of cocaine dose (0, 10, 20, and 30 mg/kg, i.p.) and time (first 60 min. session divided into twelve 5-min bins). Dependent measures included food intake, water intake, and total distance traveled in the entire chamber and rearing in food, water and control zones as well as in non-zone areas. Subsequent separate repeated measures three-way analyses of variances (ANOVAs) were computed for each dependent measure to test within group differences and between group differences. Significant findings (difference probabilities that are ≤ 0.05) warranted post-hoc comparisons that were not adjusted for family-wise error (see Keppel, 1991). The doses of cocaine were tested for significance using linear trend analyses to determine the overall nature of the drug effect. Only rats that had a non-zero baseline food intake during Experiment 1 were included in the final analyses (n = 27, CON = 8, DR = 6, MIX = 7, DIO = 6).

Results

Classification of Rats

Nine rats were randomly assigned to the CON group at the start of the study and remained on the chow pellet diet throughout the study. The remaining rats were assigned post hoc to either DIO, DR or MIX groups, after they were placed on the high-fat diet for 14 weeks. After 4 weeks time, the top nine weight gainers and bottom nine weight gainers were assigned to DIO and DR groups, respectively. The middle nine weight gainers were subsequently assigned to the MIX group (refer to Table 1). The data in this study were subsequently analyzed according to these group assignments.

Table 1: Body weight gains in DIO, DR and MIX groups after high-fat diet exposure

Group	n	Body Weight Gain (mean (g) $\pm$ sem)
DIO	9	174 ± 6.5
MIX	9	104 ± 1.6
DR	9	84 ± 4.0

Food Intake

In this study, rats were adapted to the activity chambers on each of three days prior to the assessment of cocaine on food intake, water intake and locomotion. Each chamber was outfitted with food pellets attached to the top of the front left corner and water sipper tubes extending through the lid of the chamber. Baseline food intakes after injection of vehicle averaged nearly 4 g/ first 60 min and 6 g/120min. No significant baseline food intake differences were detected between the CON, DIO-prone, DR-prone or MIX-prone groups at any time periods (first hour, second hour, and total two hours test sessions). Administration of cocaine (0,10, 20 and 30 mg/kg, i.p) in rats produced a dose dependent decrease in food intake over the first hour (Panel A, Figure 3), the second 60 min trial (Panel B, Figure 4), and the two hour ingestive trial intervals (Panel C, Figure 3) in each of the 4 groups (linear trend analyses, p's < 0.0001). Administration of 10 mg/kg cocaine induced significant decreases in food intake during the first 60 min test trial in all groups except for the MIX group (p's < 0.04), while administration of 20 and 30 mg/kg cocaine induced near-maximal suppression of food intake during the first 60 min test trial. Between-group analyses revealed no significant differences after administration of

10 mg/kg cocaine during all of the test trials. Since doses in excess of 10 mg/kg cocaine produced near-total suppressions of eating in all groups, subsequent analyses focused on this dose.

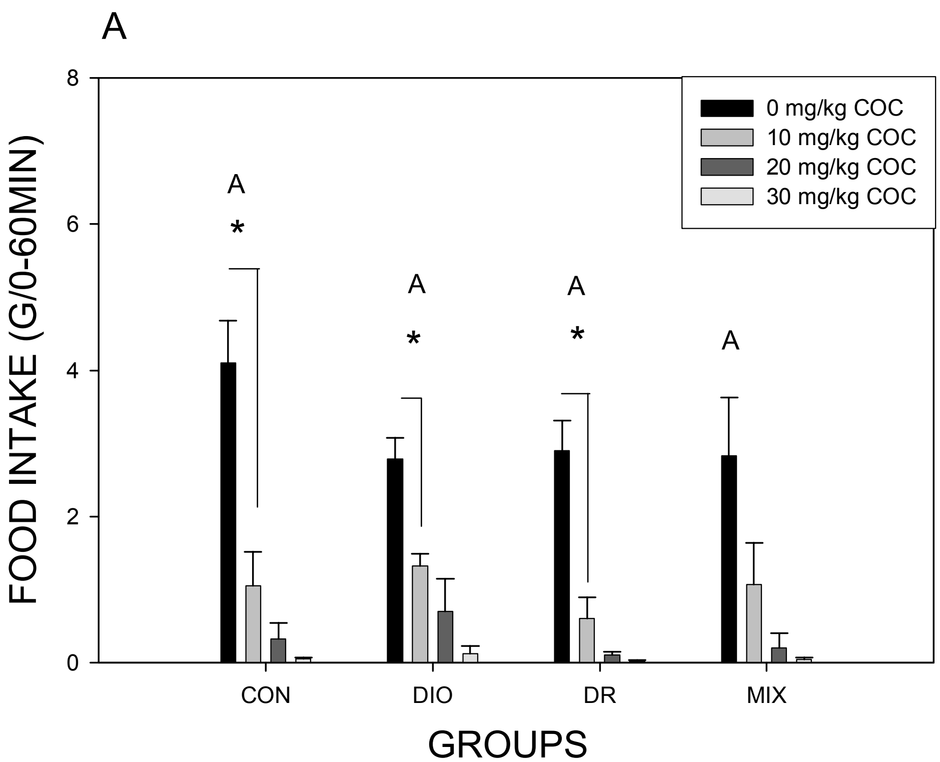


Fig. 3: Mean ($\pm$ S.E.M) group (CON, DIO, DR, and MIX) food intake during the first 60 minute test session (Panel A), second 60 minute test session (Panel B), and total 120 minute test session for rats injected with 0, 10, 20 and 30 mg/kg cocaine (i.p.; Panel C) in Experiment 1. The lines above each bar represent the S.E.M. An asterisk indicates a significant difference between doses to which arrows are pointing within a group (* = $P < 0.05$, ** = $P < 0.001$). The letter A indicates a significant linear trend for that group ($p < 0.05$).

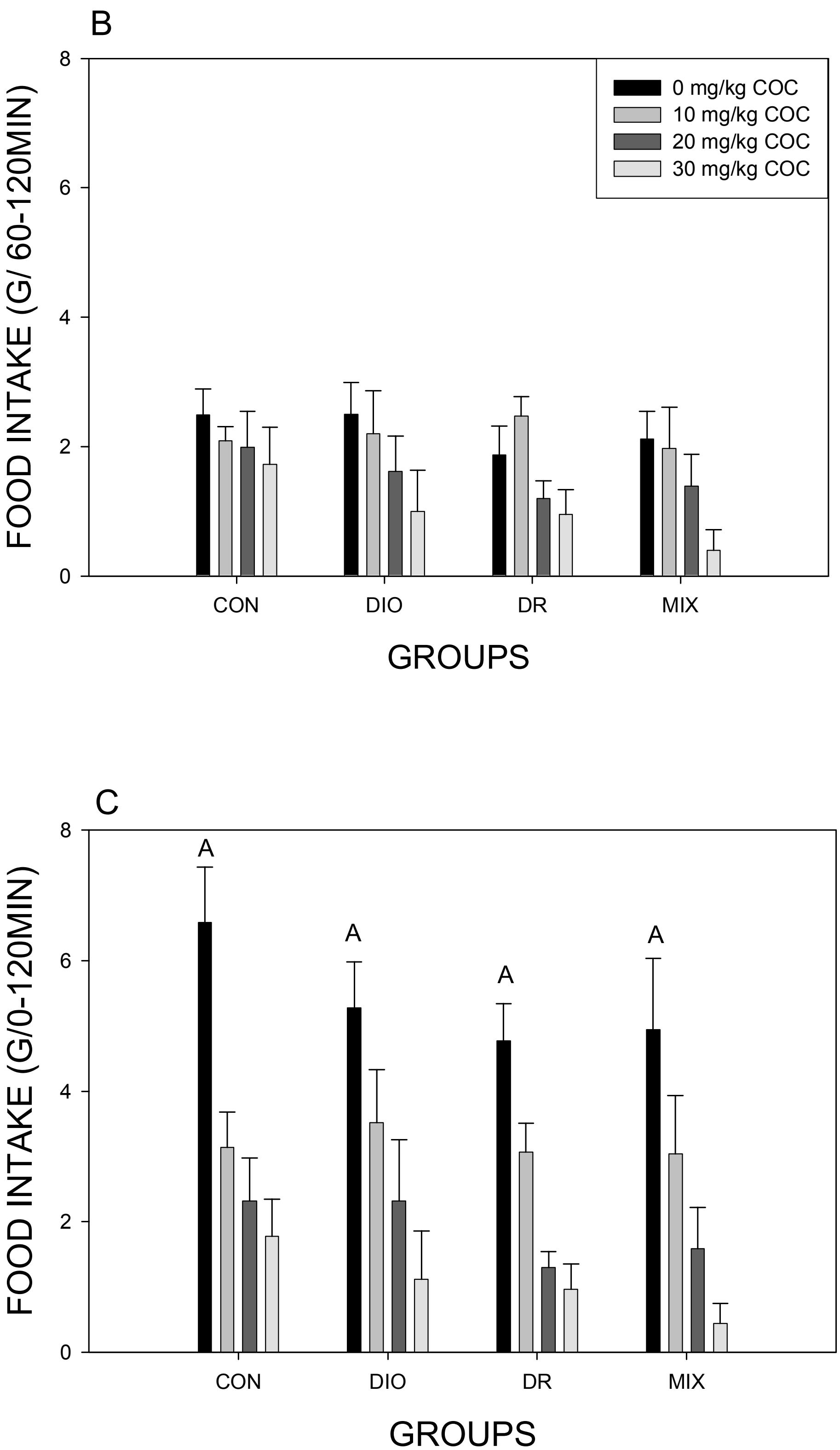


Fig. 3 continued

Water Intake

Rats had access to water via sipper tubes that extended through the lid of the activity chambers. Separate three-way ANOVAS indicated that administration of cocaine (0, 10, 20 and 30 mg/kg) produced a dose dependent decrease in water intake in all groups during the first hour (Panel A, Figure 4) and second hour (Panel B, Figure 2) as well as the total 2-hour intake period (Panel C, Figure 4; $p = 0.0001$, $p = 0.033$ and $p = 0.0001$, respectively). During the first 60-minute ingestive period, there was a baseline difference between the CON group and the other groups (DIO, DR and MIX groups), with the CON group drinking almost twice as much as the other groups (mean = 5.2 ml, p's ≤ 0.019). Linear trend analyses revealed that only CON and DR groups showed a statistically significant linear dose effect during the first hour drinking period ($p = 0.004$ and $p = 0.045$, respectively).

During the second hour drinking period, overall analyses indicated a dose effect ($p = 0.0001$), as well as a group by dose interaction ($p = 0.033$). Further analyses revealed that only the MIX group showed a statistically significant linear dose effect ($p = 0.03$). Additionally, group by dose interactions existed between the CON group and each of the other groups (DIO, DR and MIX groups; p's < 0.05). After a total of 120 min had elapsed from time of injection, the dose dependent decrease in water intake was still evident in all groups (p's < 0.04), although there were no main effects among groups, nor were any group by dose interactions observed.

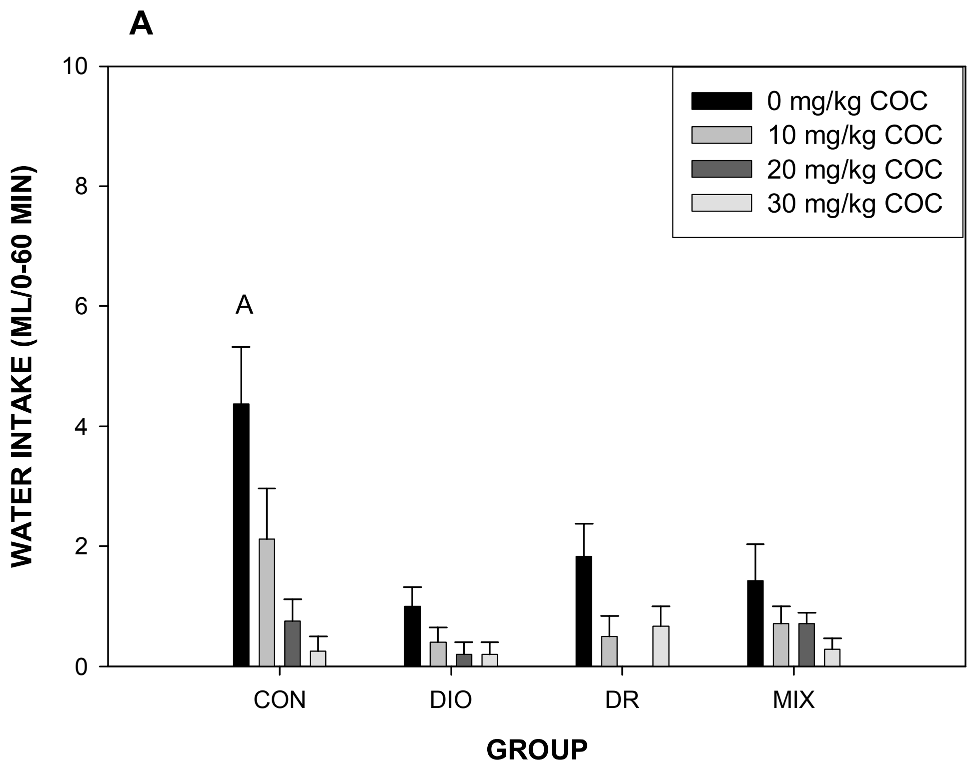


Fig. 4: Mean (± S.E.M) group (CON, DIO, DR, and MIX) water intake during the first 60 minute test session (Panel A), second 60 minute test session (Panel B), and total 120 minute test session for rats injected with 0, 10, 20 and 30 mg/kg cocaine (i.p.) in Experiment 1. The lines above each bar represent the S.E.M. The letter A indicates a significant linear trend for the group ($p < 0.05$).

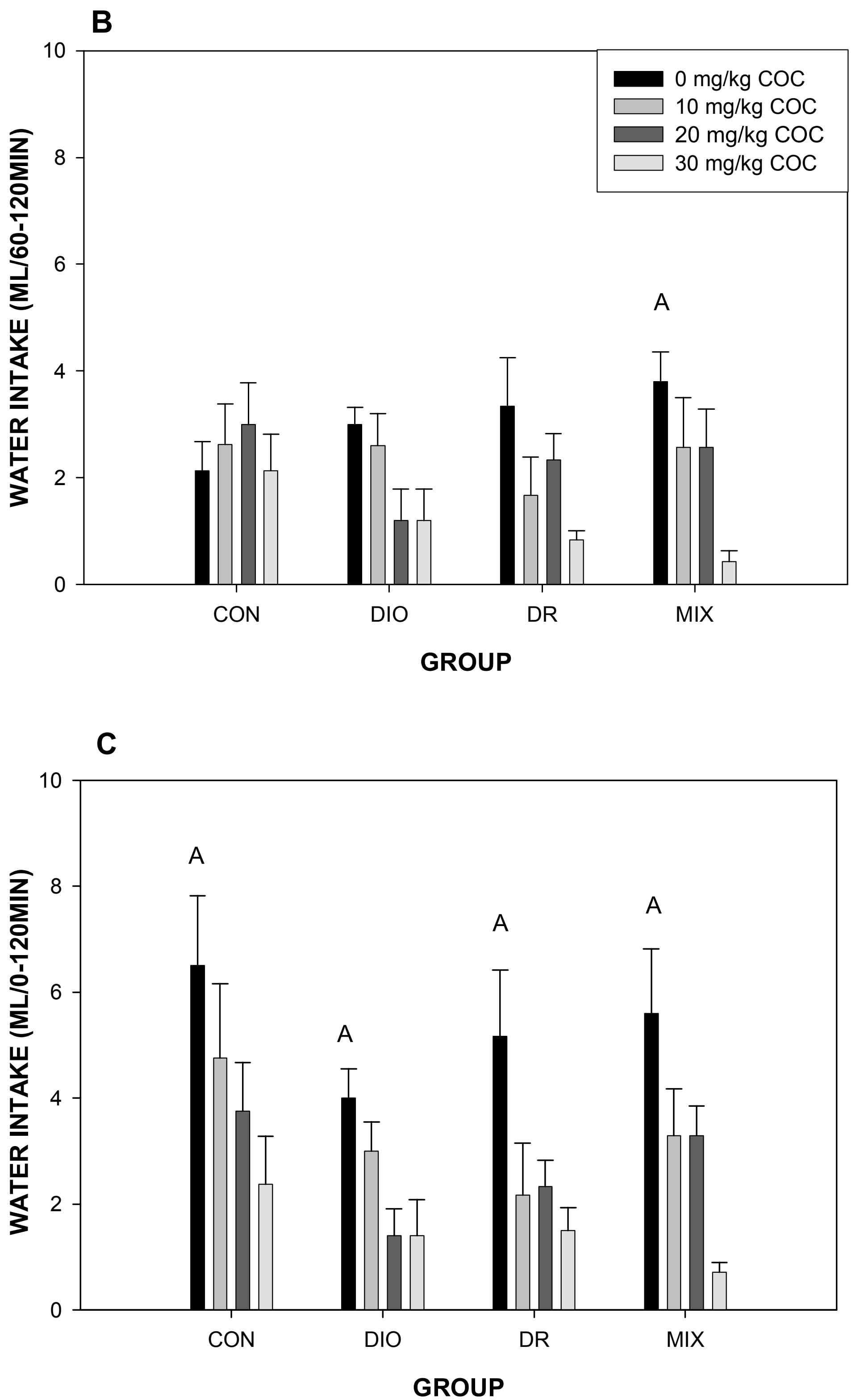


Fig. 4 continued

Locomotion

Total Distance Traveled Scores

Each test trial consisted of a 15-minute adaptation period to the locomotion chambers, after which rats were injected and returned to the chamber for the next 120 min. Expected cocaine- induced hyperlocomotion (Wellman et al., 2003; Berthold et al., 1992) was assessed in locomotion chambers for 60 min immediately after injection (time 1-12; Panel A, B, C and D, Figure 5) only since the general profile of cocaine action occurred within the first 60 min (peak at time = 1 or 2 followed by a gradual decline in activity). Activity during the 5 min. prior to injection was used to assess baseline differences between groups. Rats treated with vehicle exhibited a decline in total distance traveled scores over the first 60 min session, while administration of 20 and 30 mg/kg cocaine produced statistically significant dose dependent increases in total distance traveled over the 60 min session compared to vehicle, but were not different from each other in all groups (overall effect of dose, $p = 0.0001$). Subsequent multiple comparisons revealed that administration of 10 mg/kg cocaine produced a statistically significant increase in total distance traveled scores in DR-prone rats only (Panel B, Figure 5; $p = 0.033$). Although it appears that the DIO-prone group showed significant increases in total distance traveled scores after injection of 10 mg/kg cocaine, the apparent difference is accounted for by the elevated baseline of this group at time = 0 as compared to the vehicle group at time = 0 (Panel C, Figure 5). During the last 5 min. of the first 60 min. session, increases in total distance traveled scores after administration of 30 mg/kg cocaine remained above baseline in all groups. It is noteworthy to mention that administration of 20 mg/kg cocaine remained statistically above baseline at time 12 in

DR group only (p = 0.04; Panel C, Figure 5), suggesting increased sensitivity to cocaine's stimulant effects in the DR-prone group. There were no other statistical differences between groups during the first 60 min session.

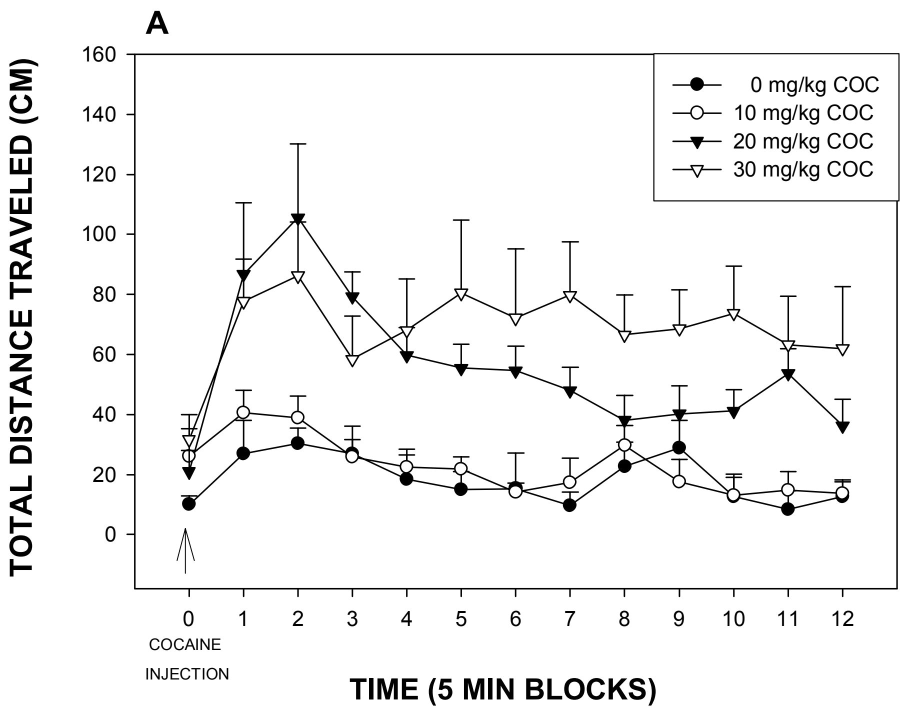
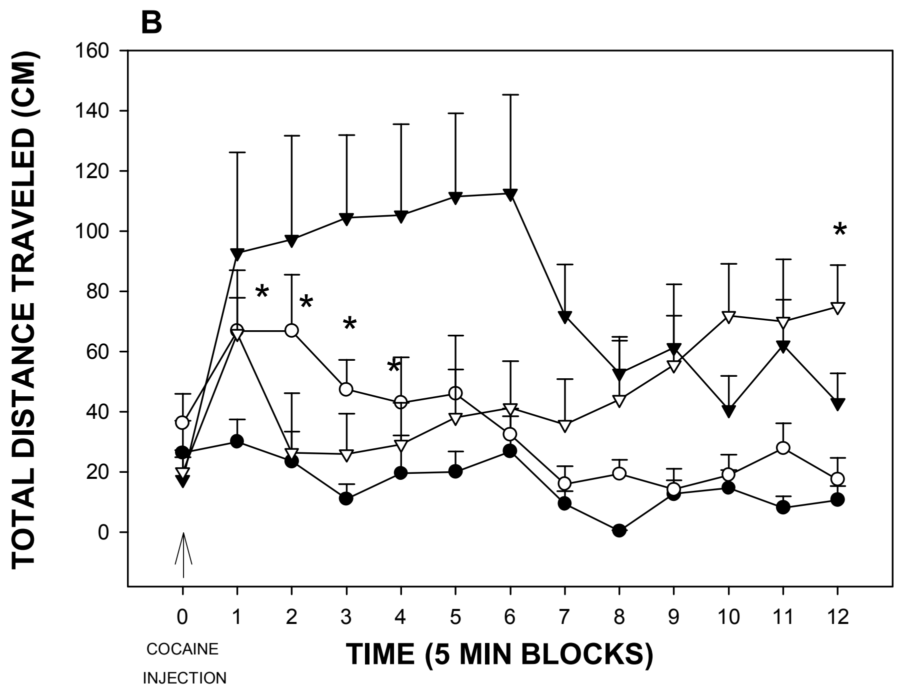


Fig. 5: Mean group (CON, DIO, DR or MIX) total distance scores (cm) in successive 5 min bins during a 5 min baseline period (Time = 0) and during a 60 min period (Time = 1-12) after injection with 0, 10, 20, and 30 mg/kg cocaine in Experiment 1. Panels A, B, C and D present total distance traveled scores for CON, DIO, DR and MIX groups, respectively. The lines above each symbol represent the S.E.M. An asterisk indicates a significant difference between the indicated dose and vehicle at the specified time point ($p < 0.05$).



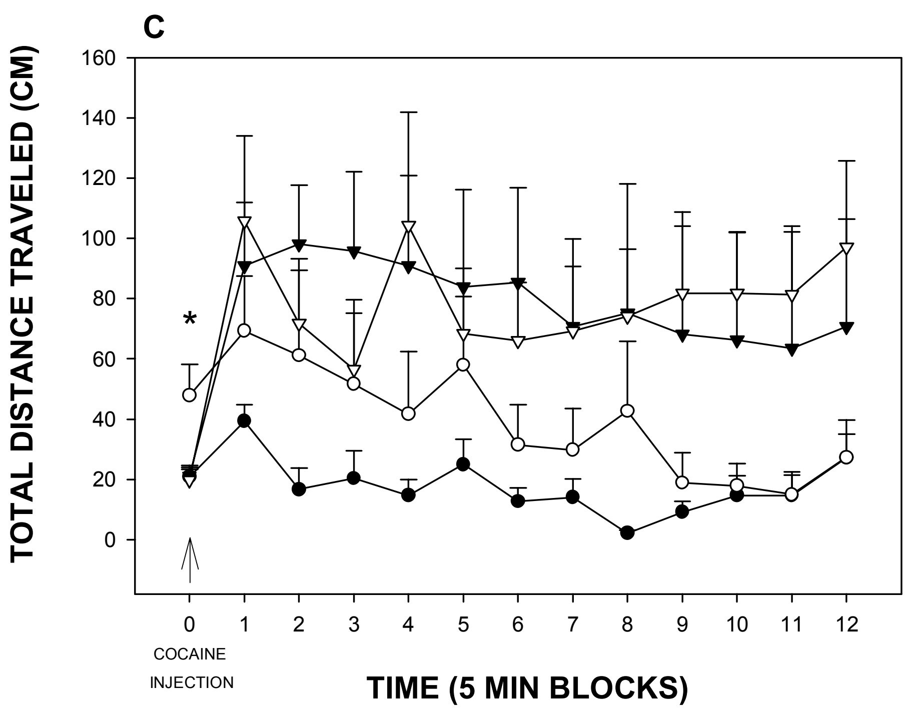


Fig. 5 continued

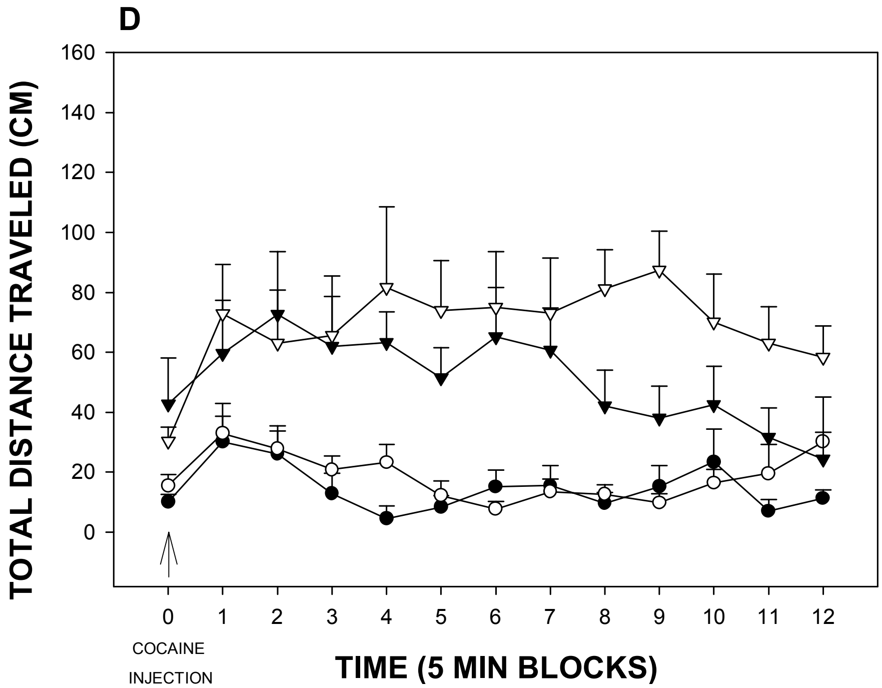


Fig. 5 continued

Rearing Scores

Rearing has been previously used as a measure of the effects of cocaine on locomotion. For this study, vertical activity scores reported by Versamax were analyzed according to food and water zone versus the corresponding control zones (see Figure 2) in order to assess the relation between feeding-related (food and water zones) rearing and cocaine-induced rearing (control zones). Analyses comparing the zones indicated no correlation between feeding-related rearing and cocaine-induced rearing. It was hoped that consideration of rearing in the food zone would provide key information as to the temporal aspect of feeding; but this aspect was not suitable for analysis.

Although the total rearing scores over the first 60 min, second 60 min and 120 min test sessions (data not presented for any test session) revealed a step-wise increase in rearing as cocaine dose administered increased, this trend only reached significance in the MIX group ($p = 0.0001$; data not presented). Within the MIX group, rearing scores did not increase after administration of 10 mg/kg cocaine over the 60 min. test session. In contrast, administration of 20 mg/kg cocaine did significantly increase rearing over the first half of the 60 min session, while rearing scores after administration of 30 mg/kg remained significantly different from baseline and 10 mg/kg cocaine dose over the entire 60 minute period (p's ≤ 0.046). The groups were not significantly different from each other at any dose.

EXPERIMENT 2

Materials and Methods

Animals

The rats of Experiment 1 were retested for cocaine reactivity in Experiment 2, in order to assess cocaine hypophagia and cocaine hyperlocomotion in rats after exposure to a high-fat diet resulting in differential weight gains (depending on genetic predisposition to obesity). Due to the limited number of locomotor chambers, half of the rats in each squad remained under the illumination schedule with lights off at 1500h while the other half were adapted to a schedule of lights off at 12 pm. This regimen allowed for each rat to be tested at the start of the dark cycle. They remained in their respective squads for the length of the study. In Experiment 2, rats were injected again with three doses of cocaine and thus these rats are not considered to be cocaine-naïve. Whether these repeated treatments were associated with sensitization or tolerance would be estimated by the changes in reactivity to cocaine noted in CON group from Experiment 1 to Experiment 2.

High-Fat Diet

The diet consisted of one-part vegetable shortening and two-part ground rat chow (Teklad, 8728c; Cooper, 1987). The vegetable shortening was heated in a large glass mixing bowl until completely melted and the chow was added and mixed thoroughly. The rats were offered approximately 60g of this diet in a glass custard dish (300 ml) that was washed and refilled with a fresh batch of diet every 48 hrs. This diet is proven to produce variable body weight gains in rats as seen in a pilot study conducted by this lab as well as Levin's studies (1997, 1996, 1986).

Procedure

At the start of the study, nine rats were randomly assigned to the control group (CON) and were maintained on chow pellet diet for the remainder of the study; the rest of the rats were placed on a high-fat diet (as described above). After 4 weeks on the high-fat diet, the top 9 weight gainers were assigned to the DIO group, the lowest weight gainers were assigned to the DR group, and subsequently, the residual weight gainers were assigned to the MIXED group. This method of assignment is that used by Levin (1986; 2000) to differentiate DIO from DR rats. Both DIO and DR groups continued on the high-fat diet for an additional 45 days while the MIXED group was maintained on standard chow for the remainder of the study. At the end of the 45 days, both DIO and DR groups were switched back to the chow pellet diet, and all rats were maintained on standard chow for 2 weeks, after which testing commenced. The CON group allowed for the assessment of a possible, sensitization or tolerance of the hypophagic and hyper-locomotor actions of cocaine during the second time exposure to cocaine (Davidson et al., 2002; Georgetti and Zhdanova, 2000). Testing procedures were the same as that in Experiment 1 except that in Experiment 2, because of the remarkable increase in the size of the rats, the dividers were removed to allow for the testing of one rat per chamber (see Figure 6).

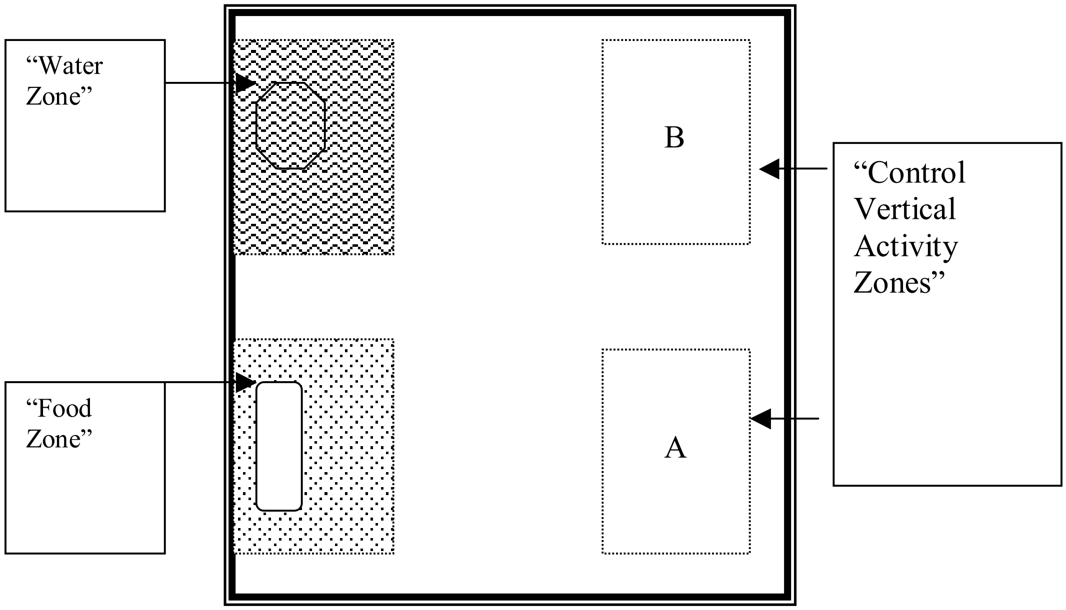


Fig. 6: A schematic of the locomotor activity chamber used to assess food intake and locomotor activity during the Experiment 2. The view is from the top of the chamber looking down onto the chamber floor. Each chamber is 40 X 40 X 30.5 cm with food, water and control zones each measuring 5.08 X 5.08 X 30.5 cm.

Data Analyses

Data analyses were similar to that computed for Experiment 1. Comparison of regression lines characterizing the relationship between body weight gain and cocaine hypophagia and cocaine hyperlocomotion in Experiment 1 and 2 were made using methods provided by Ferguson (1981). Only rats that had a non-zero baseline intake during Experiment 2 were included in the analyses (n = 28; CON = 9, DR = 6, MIX = 5, DIO = 8).

Results

Body Weight Gain in Rats

Prior to high-fat diet exposure, the groups did not differ in terms of mean group body weight (Figure 7). After all rats except for CON group were placed on a high-fat diet for only one week, the DIO group weighed significantly more than both the CON and DR groups (p's < 0.002). It was not until the end of week 4, that MIX rats became significantly different from DIO rats ($p = .002$). At the time that the MIX group was put back on the chow diet, their body weight curve differed only from the DIO group ($p = 0.0001$). As expected (Levin and Dunn-Meynell, 2000), the DIO group remained significantly heavier than the rest of the groups for the remainder of the study, even after DIO and DR groups were placed back on chow for the 2 weeks prior to cocaine test sessions in the second study (p's < 0.0001).

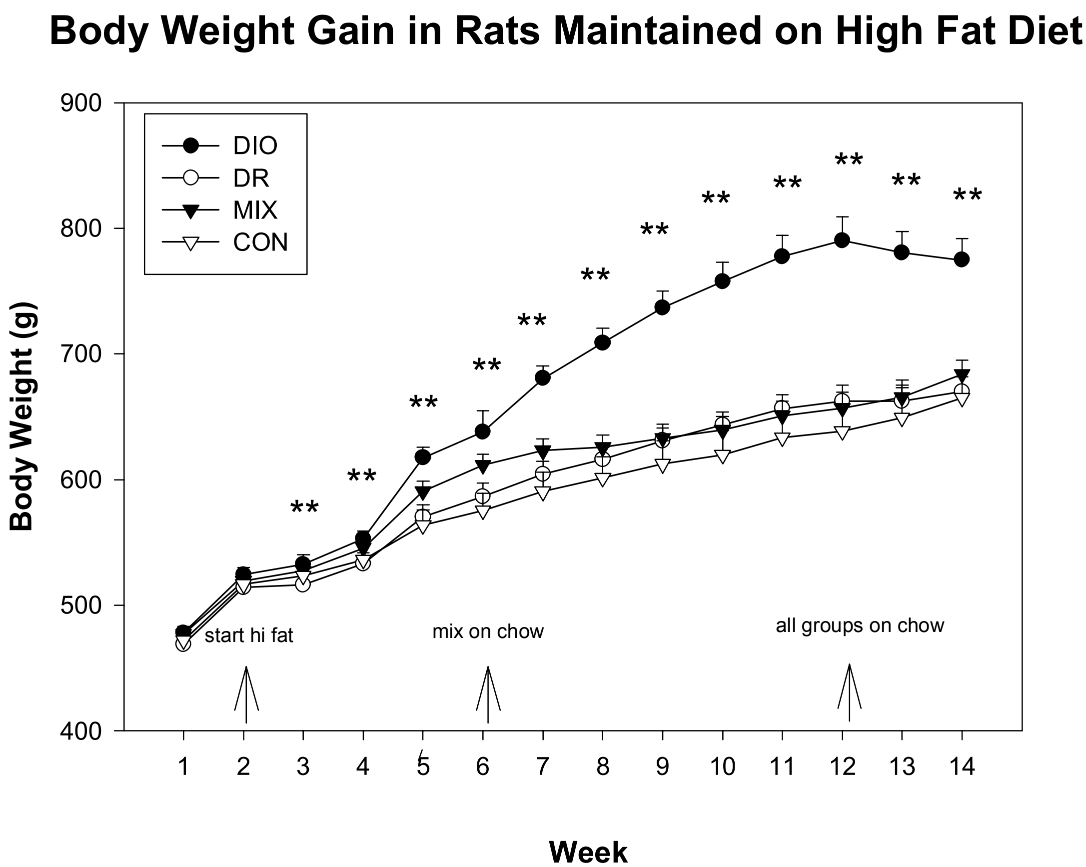


Fig. 7: Mean group body weights (g) over a 14 week period. The arrows indicate diet regimen for all groups except for the CON group, which remained on the chow diet throughout the entire study. The lines above each symbol represent the S.E.M. An asterisk indicates a significant difference between the DIO group and the other groups (* = p<0.05, ** = p<0.01).

Food Intake

In this study, the rats were placed back on the pellet chow diet 2 weeks prior to the start of the ingestive trial sessions in the activity chambers. There were no differences among the groups after injection of vehicle during the first 60 min, second 60 min, and 120 min test sessions.

Simple comparison analyses indicated that the dose response curve for 60 min food intake in all groups was significantly different from zero (ANOVA, p's $\leq$ 0.007). Administration of 10 mg/kg cocaine caused a significant reduction in food intake for CON, DIO and DR groups but not for the MIX group (p's < 0.04), suggesting that the MIX group is less sensitive to cocaine hypophagia. In all groups, administration of 20 and 30 mg/kg cocaine produced a near-maximal reduction in food intake. Notably, after administration of 10 mg/kg cocaine, the DIO group showed a marked decrease in food intake that was significantly lower than both CON, and MIX groups given the same dose (p = 0.050, p = 0.034, respectively).

Analyses of the second 60 min and 120 min test session revealed a significant overall linear dose response curve with additional comparisons revealing significant linear trends for each group (p's $\leq$ 0.05; Panel C, Figure 8). There was not, however, an overall group by dose interaction at either test session (p = 0.060). Despite the lack of an overall interaction, administration of 10 mg/kg cocaine produced a decrease in food intake during the first hour test session in DIO group whose magnitude was greater than that seen in both CON and MIX groups. The percent decrease in food intake after administration of 10 mg/kg cocaine was 83 % in the DIO group, while after injection of the same dose, CON, DR and MIX groups ate about 52 %, 61% and 25% less than baseline, respectively.

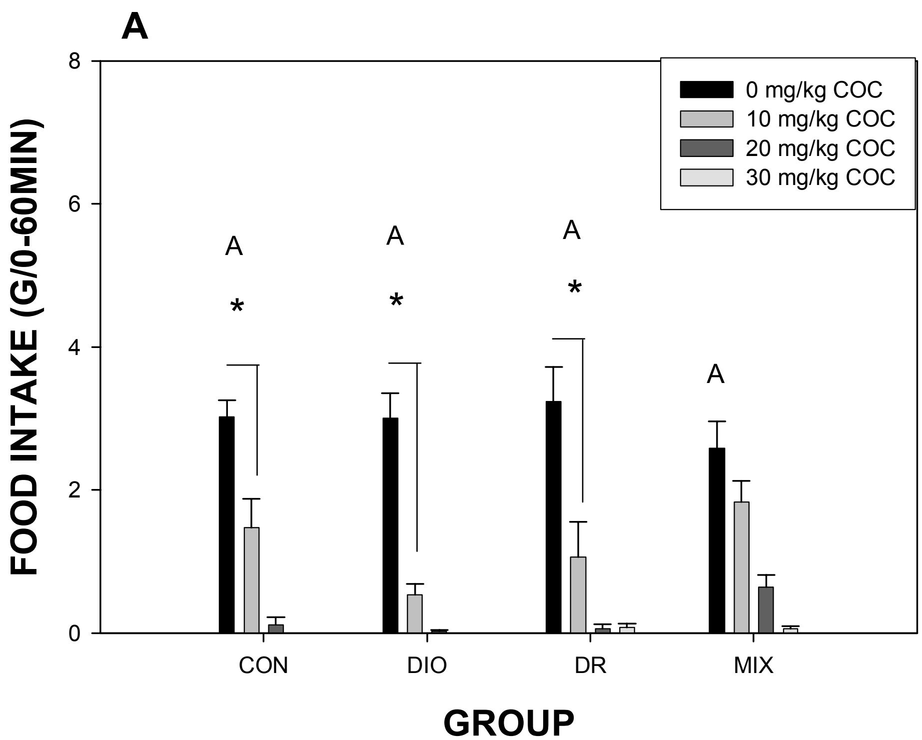


Fig. 8: Mean (± S.E.M) group (CON, DIO, DR, and MIX) food intake during the first 60 minute test session (Panel A), second 60 minute test session (Panel B), and total 120 minute test session for rats injected with 0, 10, 20 and 30 mg/kg cocaine (i.p.) in Experiment 2. The lines above each bar represent the S.E.M. An asterisk indicates a significant difference between doses to which arrows are pointing within a group (* = $P < 0.05$, ** = $P < 0.001$). The letter A indicates a significant linear trend for that group ($p < 0.05$).

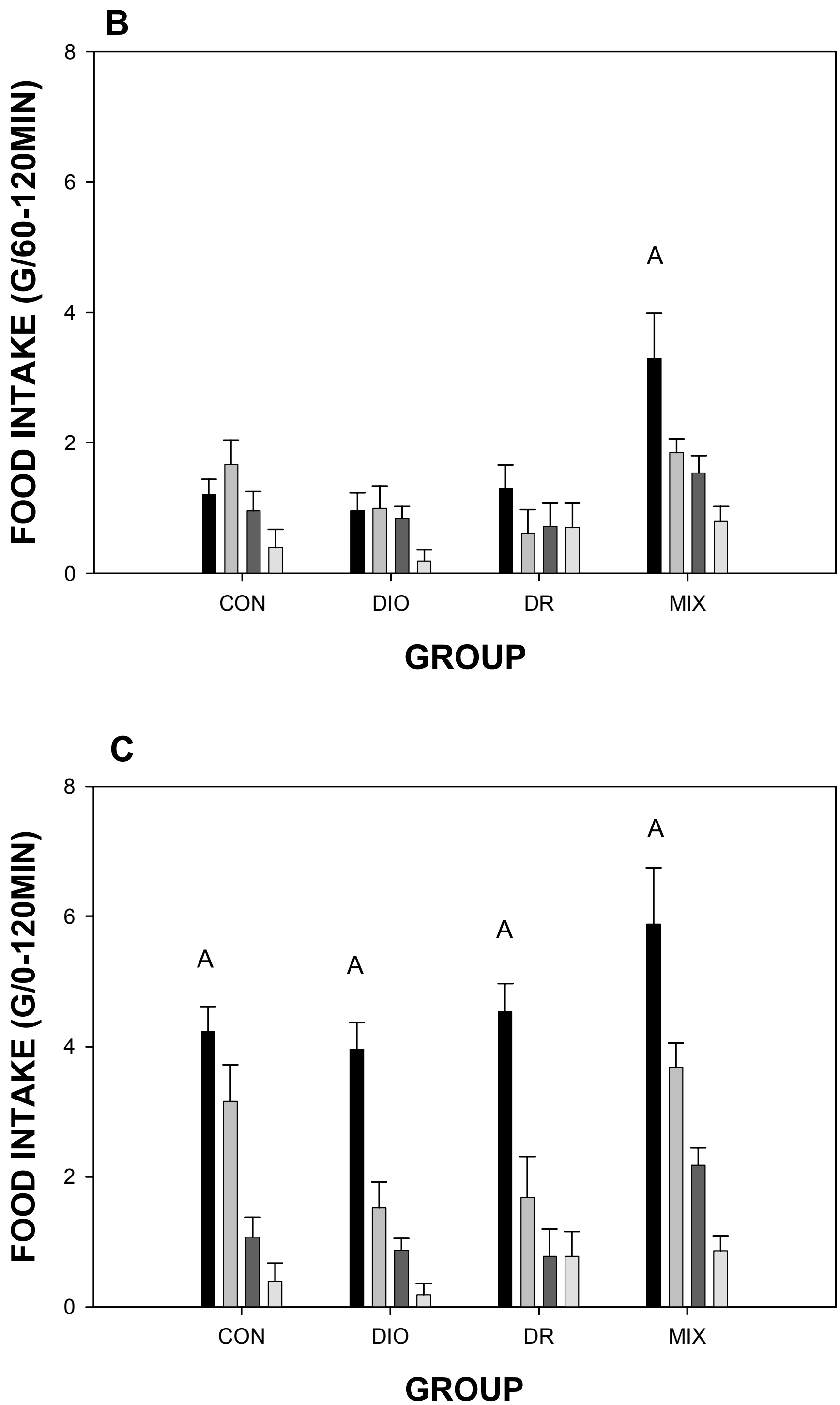


Fig. 8 continued

Water Intake

Administration of cocaine (0, 10, 20 and 30 mg/kg) produced a dose dependent decrease in water intake during the first 60 min (Panel A, Figure 9) and second 60 min (Panel B, Figure 9) as well as the total 120 min test period (Panel C, Figure 9) in terms of dose per se (p's ≤ 0.02). Although this suggests that water intake could be a byproduct of the suppression of food intake, closer inspection of the hypodypsic effect of cocaine after administration of 10 and 20 mg/kg cocaine did not produce the same magnitude of suppression that was observed for cocaine hypophagia. During the first 60 min intake session, administration of 10 mg/kg and 30 mg/kg cocaine produced marked hypodypsia ($p = 0.04$ and $p = 0.023$, respectively). During the second 60 min intake session, cocaine-induced hypodypsia reached significance from baseline after 30 mg/kg cocaine in CON group and nearly reached statistical significance in DIO group ($p = 0.04$ and $p = 0.06$, respectively). Analyses of water intake during the total 120 min test session indicated a decrease in water intake in CON group after administration of 10, 20 and 30 mg/kg cocaine ($p = 0.02$, 0.02, and 0.01, respectively).

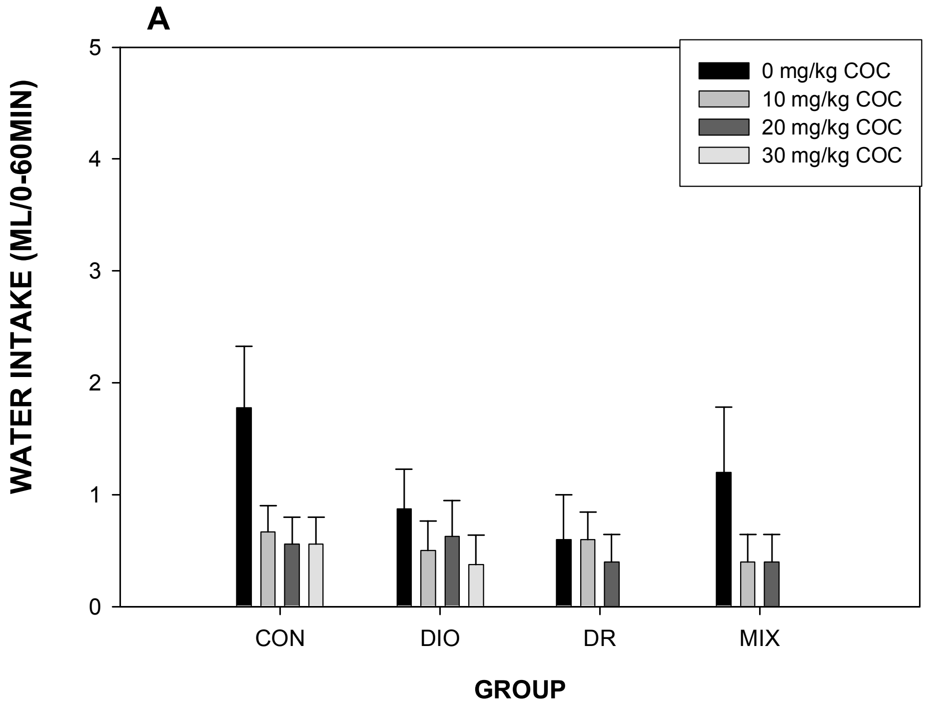
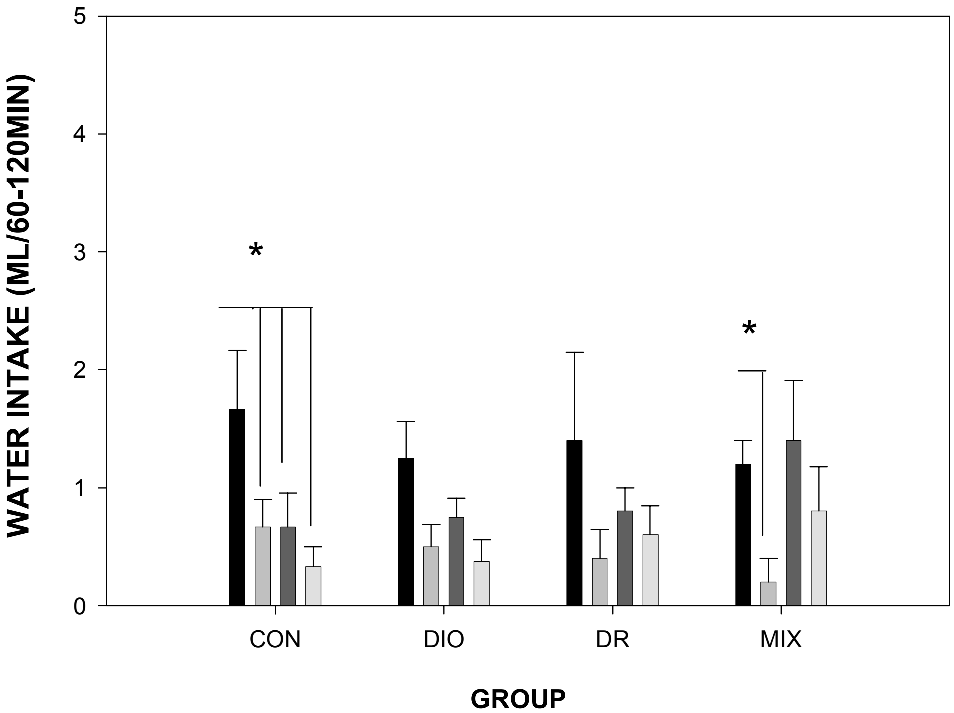


Fig. 9: Mean (± S.E.M) group (CON, DIO, DR, and MIX) water intake during the first 60 minute test session (Panel A), second 60 minute test session (Panel B), and total 120 minute test session for rats injected with 0, 10, 20 and 30 mg/kg cocaine (i.p.) in Experiment 2. The lines above each bar represent the S.E.M. There was an overall effect of dose for each test session, but post-hoc comparisons revealed no significant difference between doses, except in the CON and MIX groups (asterisk indicates significant differences between dose ($p < 0.05$)

B



C

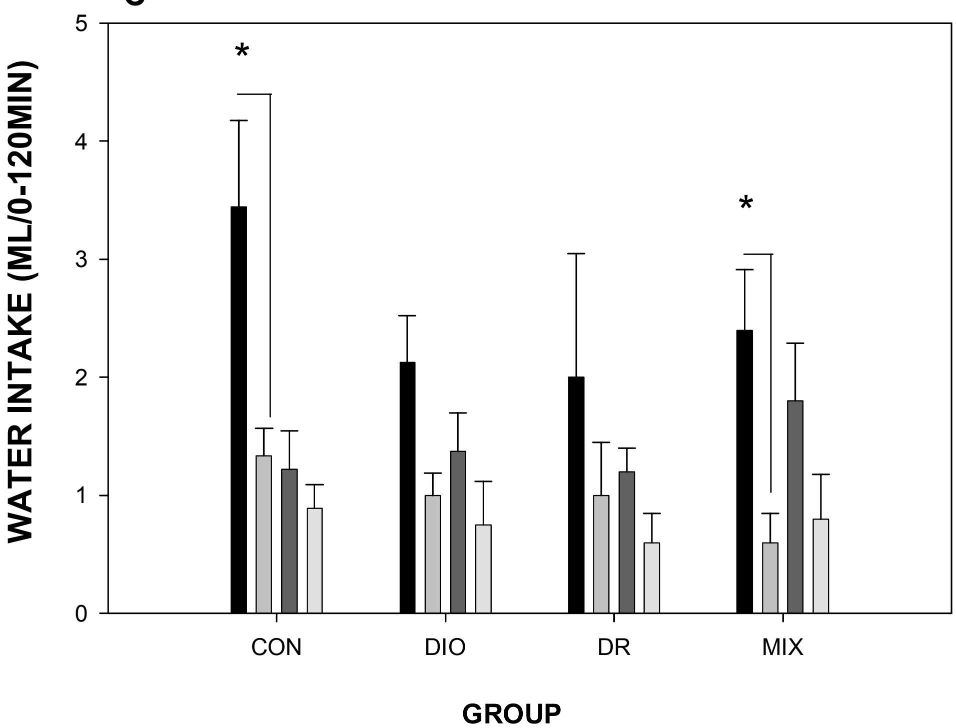


Fig. 9 continued

Locomotion

Total Distance Traveled Scores

Locomotion was assessed in the activity chambers used in Experiment 1 except that each rat had access to the entire chamber as opposed to half the chamber. Each trial consisted of 15 minutes adaptation to the locomotion chambers, after which rats were injected and returned to the chamber for the next 120 min. Because the general profile of cocaine action occurred within the first 60 min (peak at time = 2 followed by a gradual decline in activity), analyses were performed for the first 60 min only using 5 min bin intervals (Panel A, B, C, and D, Figure 10). Activity during the 5 min prior to injection was used to assess baseline differences between groups and between subsequent 5 min bins. Rats injected with vehicle at time = 1 showed a decline in total distance traveled with activity during time 3 – 12 equal to nearly 0 cm in all groups. In contrast, administration of 10, 20 and 30 mg/kg cocaine produced dose-dependent increases in total distance traveled scores over 60 min test session in all groups (p's < 0.05). Administration of these doses (10, 20, and 30 mg/kg) produced a peak in total distance traveled scored during the first 5-15 min (time 1 – 3) after injection, after which a gradual decline ensued. In all groups, at the end of the 60 minute session (last 5 min), total distance traveled after administration of 10 mg/kg cocaine returned to baseline while total distance traveled scores after 20 and 30 mg/kg cocaine remained significantly elevated. Injection of 10 mg/kg cocaine in the DIO rats produced an increase in total distance traveled that remained significantly different from baseline from time 1 to time 11 (p's < 0.05; Panel B, Figure 10), while injection of the same dose in the CON, MIX and DR rats returned to baseline levels at time 7 or 8 (p's < 0.05; Panel A, C, and D, Figure 10).

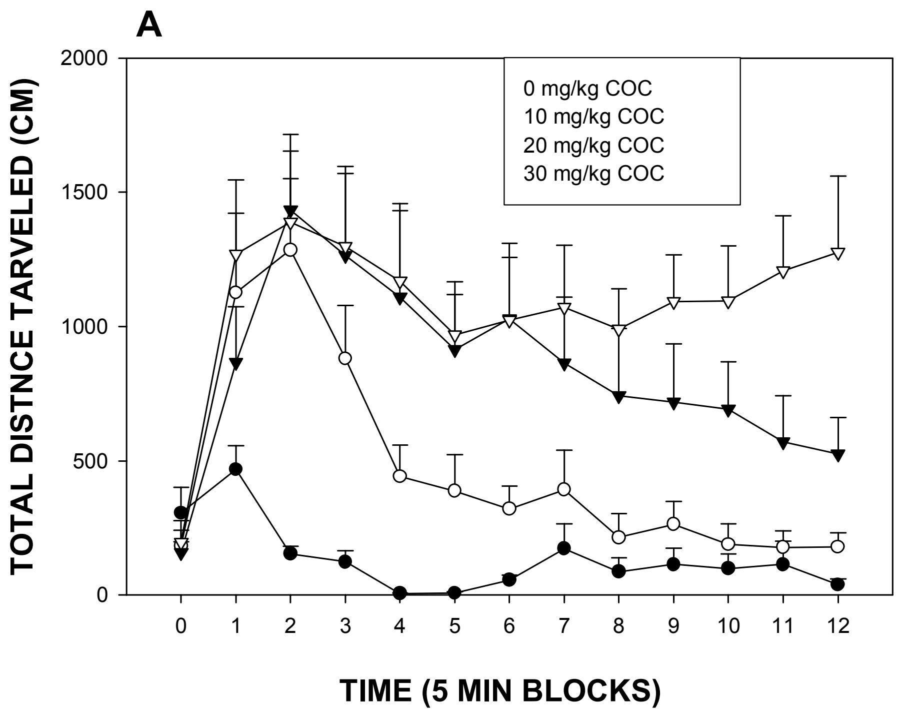


Fig. 10: Mean group (CON, DIO, DR or MIX) total distance scores (cm) in successive 5 min bins during a 5 min baseline period (Time = 0) and during a 60 min period (Time = 1-12) after injection with 0, 10, 20, and 30 mg/kg cocaine in Experiment 2. Panels A, B, C and D present total distance traveled scores for CON, DIO, DR and MIX groups, respectively. The lines above each symbol represent the S.E.M. An asterisk indicates a significant difference within groups at the specified dose relative to vehicle injection at the indicated time point ($p<0.05$).

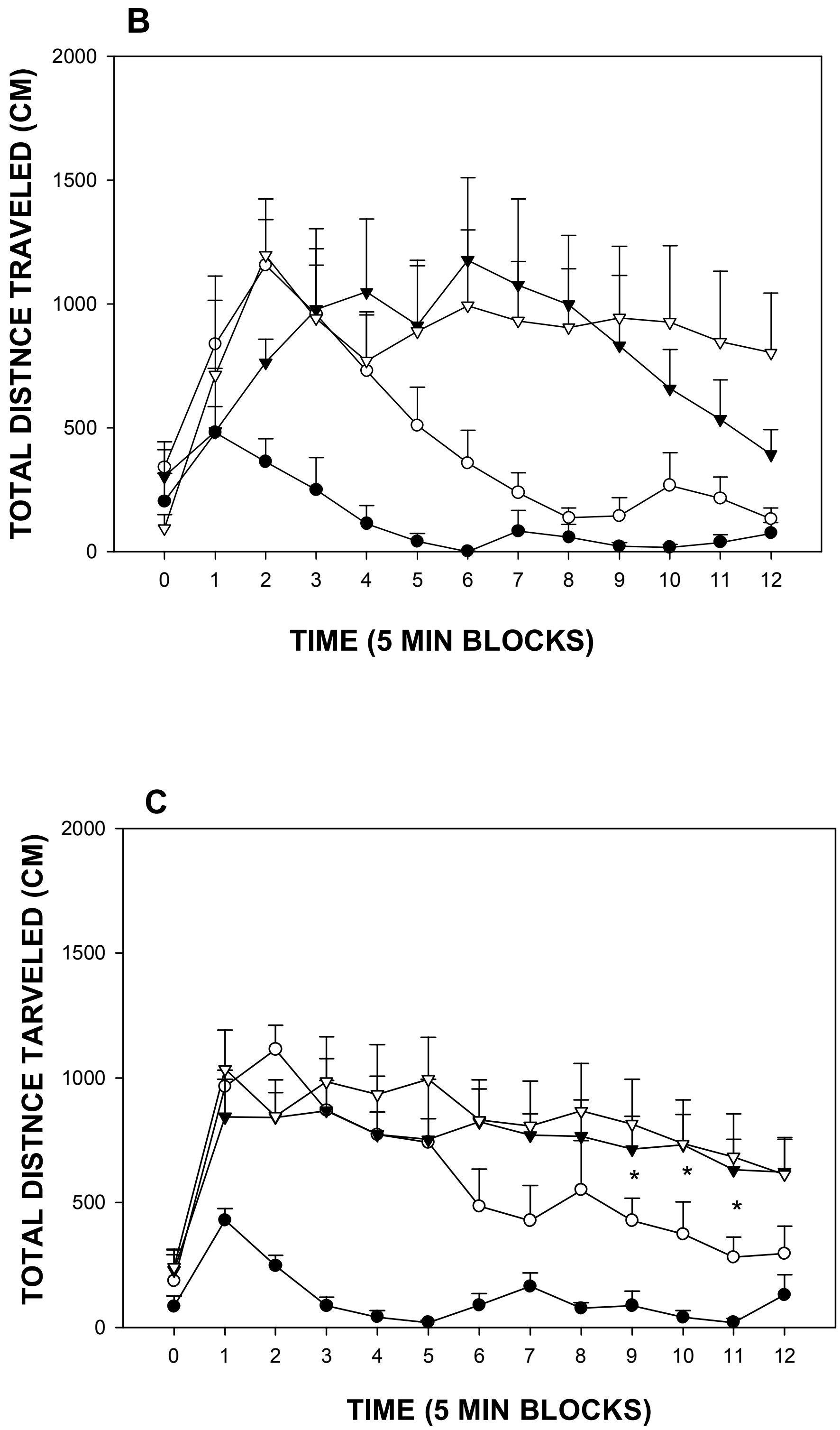


Fig. 10 continued

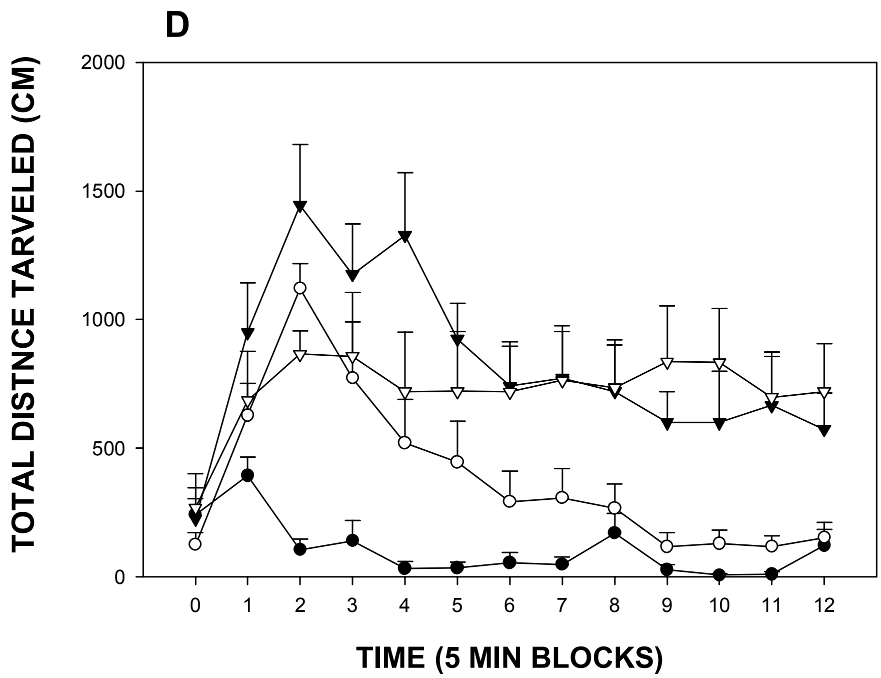


Fig. 10 continued

Rearing

Cocaine did not increase rearing in a dose dependent manner in study 2 (data not presented). There is a slight step-wise increase in rearing after injection of 0, 10, and 20 mg/kg cocaine, but injection of 30 mg/kg cocaine actually produced a smaller increase in rearing as compared to injection of 20 mg/kg cocaine. Additionally, in the DIO group, administration of 30 mg/kg cocaine produced a suppression of rearing activity. Three-way ANOVA's revealed no significant differences between the groups at any dose of cocaine.

Comparison of Experiment 1 and Experiment 2

Food intake

Regression analyses that considered each individual rats' body weight change from the start of the study to 4 wks high-fat exposure, and percent change in food intake at 10 mg/kg cocaine from vehicle allowed for an alternative strategy that treats body weight gain as a continuous variable rather than a categorical variable. Hypophagia induced by 10 mg/kg cocaine was used because 20 and 30 mg/kg cocaine produced a near maximum decrease in food intake (nearly 0). In Experiment 1, linear regression analyses indicated that rats showing the greatest percent weight gain (after the cocaine trials) showed the least degree of hypophagia after receiving 10mg/kg cocaine evident in first 60min and the 120min test sessions ($r = 0.35$ and 0.33, respectively). During the second 60 min test session (time 13 – 24), food intake among rats reflected a trend nearly close to zero.

The strategy used to examine food intake after administration of 10 mg/kg cocaine before exposure to high-fat diet was similarly applied to cocaine hypophagia in rats after exposure to high-fat diet (Experiment 2). Again, percent body weight gain during the first 4 weeks of high-fat exposure period was plotted against percent change from baseline food intake to food intake after administration of 10 mg/kg cocaine for each rat. In this second analysis using the first 60 min food intake session, rats that gained the most body weight showed the greatest hypophagic reactivity to cocaine while the lowest weight gainers showed the least reactivity ($r = .402$, $p = 0.099$). Linear regression analyses indicated a significant positive correlation between body weight change and food intake change ($r = 0.500$, $p = 0.041$), indicating increasing compensation during the second 60 min test trial as body weight gain increased. Trend for total 120min intake paralleled that seen in the 60min intake session ($r = 0.324$, $p = 0.184$).

The positive correlation between body weight gain and change in food intake after injection of 10 mg/kg cocaine from vehicle that existed before rats were exposed to the high-fat diet suggests that the regression line from Experiment 2 should not be compared to zero, but rather to the regression line of Experiment 1. Thus, the corresponding correlation coefficients were analyzed for statistical difference. Although neither 60 min food intake regression lines in study 1 nor 60 min food intake regression lines in study 2 were statistically different from zero, they were statistically different from one another (comparison of two correlation coefficients using Fisher's z scores (see Ferguson, 1981), $p = 0.05$, Panel A, Figure 11). Additionally, there were nearly significant differences between Experiment 1 and Experiment 2 during second 60 minute and 120 minute ($z = 1.52$ and $z = 1.82$, respectively).

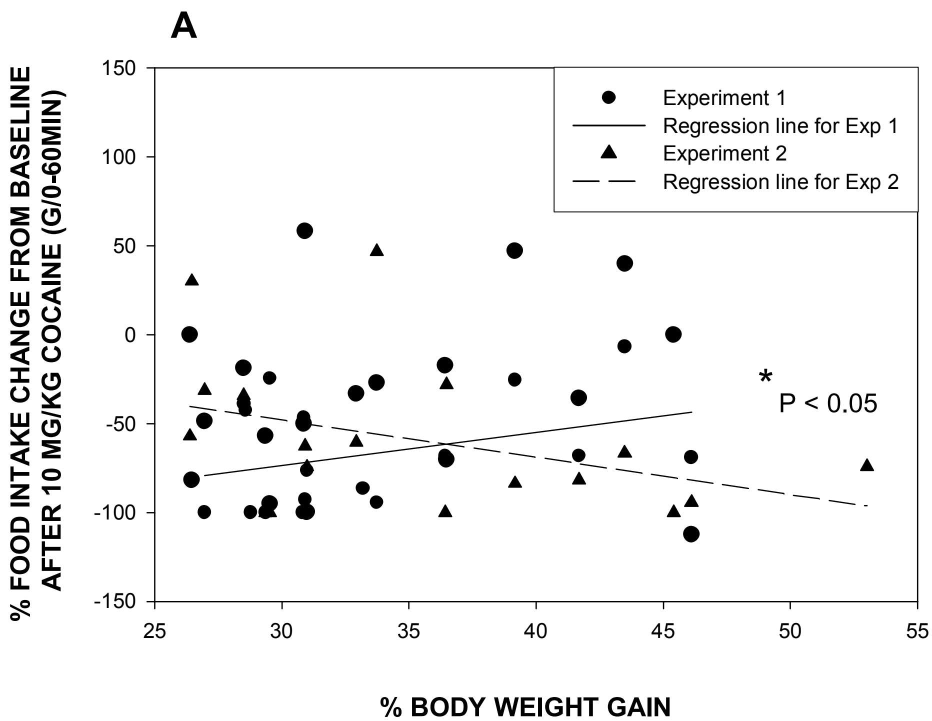
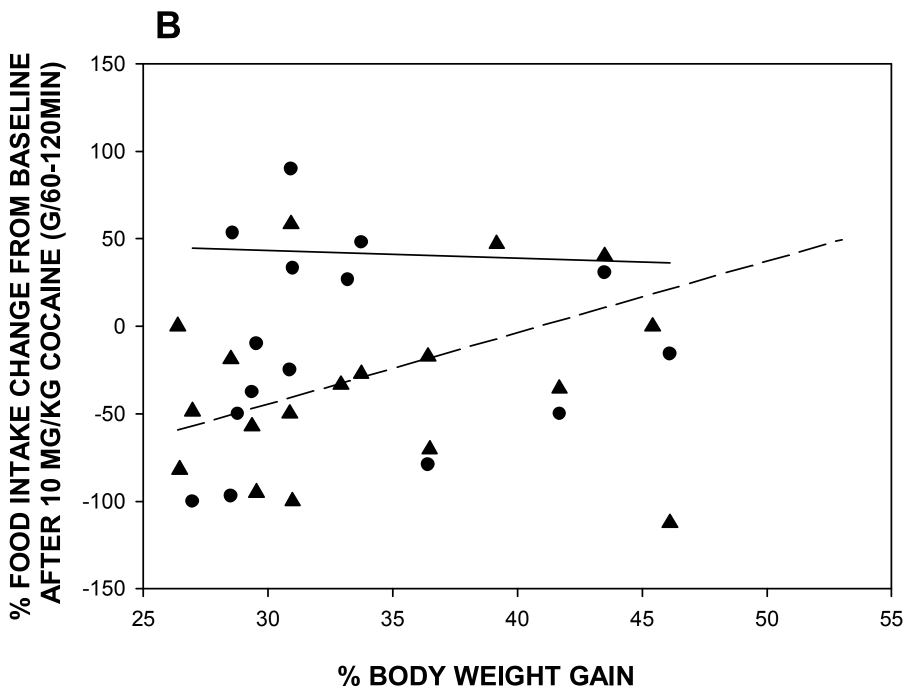


Fig. 11: A comparison of cocaine hypophagia in Experiment 1 and Experiment 2 after 10 mg/kg cocaine. Panels represent food intake after the first 60 minute test session (Panel A), second 60 minute test session (Panel B), and total 120 minute test session. Linear regression lines characterize the percent change in food intake from baseline as a function of body weight gain. Circles represent individual data points from Experiment 1 and triangles represent individual data points from Experiment 2. An asterisk indicates a significant difference between the regression lines of Experiment 1 and Experiment 2 ($p < 0.05$).



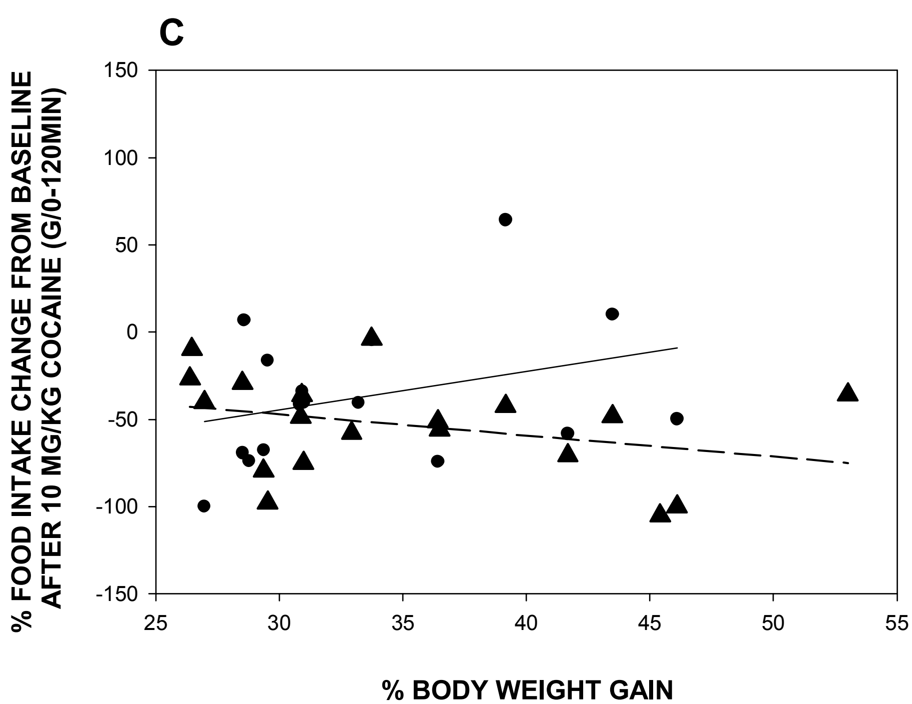


Fig. 11 continued

Water Intake

During the first and second 60 min test sessions, percent change from baseline after administration of 10 mg/kg cocaine was largely dichotomous, with the majority of the rats either showing total suppression of water intake or no change from baseline (-100% and 0% change, respectively; data not presented). Dichotomous data does not easily lend itself to regression analyses, thus analyses were performed only for 120 minute water intakes. In study 1, administration of 10 mg/kg cocaine produced a greater suppression of water intake in the lowest weight gainers as is evident in the positive regression line ($r = .412$). In contrast, in study 2, the correlation between percent body weight gain and percent change in food intake from baseline after administration of 10 mg/kg cocaine was negative ($r = -0.179$). Although these two regression lines were not significantly different from zero, they were significantly different from each other ($z = 3.01$, $p < .01$). This pattern parallels that of food intake.

Total Distance Traveled Scores

Inspection of the regression lines for study 1 and study 2 suggests a different pattern for each study (data not presented). In Experiment 1 and Experiment 2, rats did not differ in their total distance scores after administration of 10 mg/kg cocaine over the first 60 min test session or the 120 min test session. However, in Experiment 1, rats did show a notable, but non-significant, tendency to have smaller increases in total distance scores in the second 60 min test session as the magnitude of their body weight gain increased ($r = -0.321$). In contrast, in Experiment 2, the effect over the second 60 min test session was of the opposite nature. As percent body weight gain increased, percent change in total distance traveled from baseline after administration of 10 mg/kg cocaine increased as well. The trends for both studies were not significantly different from zero, but when compared against each other, they were almost significantly different ($z = 1.35$). It must be noted, however, that the comparison of total distance traveled scores in Experiment 1 and Experiment 2 should be cautiously interpreted due to the difference in chamber size between the two studies. That is, in Experiment 1, the chamber sizes were small and might be associated with restriction of locomotor activity, whereas in Experiment 2, the chamber sizes were larger. Indeed the baseline scores after vehicle were about 20 cm in Experiment 1, but were much higher in Experiment 2.

CONCLUSIONS

The influence of propensity for obesity on cocaine reactivity was considered in Experiment 1, which documented that DR-prone rats exhibited greater reactivity to cocaine than did DIO-prone rats. Moreover, Experiment 2 of the present study further considered the impact of obesity per se on reactivity to cocaine and noted that DIO rats exhibited significant enhancement of reactivity to cocaine. These findings suggest that the physiological underpinnings of resistance to obesity confer an enhanced reactivity to cocaine, which might be expected to result in greater reactivity to cocaine and perhaps exaggerated propensity to develop cocaine abuse. Moreover, the enhanced reactivity noted after the development of obesity suggests that, at a minimum, cocaine would retain its capacity to suppress appetite in an obese person and that obese persons may be at greater risk for developing cocaine abuse. The exact physiological mechanisms that may underlie these effects in DR-prone and DR rats are yet as yet unknown. In the following sections, some key findings regarding DR-prone and DIO rats are considered as possible explanations for these basic findings in the present thesis.

As expected (Levin and Dunn-Meynell, 2000), rats exposed to a high-fat diet diverged in terms of body weight allowing for the assessment of cocaine hypophagia and cocaine hyperlocomotion in DIO, DR and MIX rats both before and after body weight gain due to high-fat exposure. The maintenance of rats on a high-fat diet at the end of Experiment 1, caused significantly rapid weight gain in the DIO group, while DR and MIX groups had weight gains that were comparable to those in the chow-fed CON group. The maintenance of high body weight in the DIO group, after being put back on the chow pellet diet after 11 weeks on high-fat diet, suggested a permanent shift in body weight set point (Levin and Dunn-Meynell, 2000). The MIX group, on the other hand, showed an accelerated rate in body weight gain while maintained on the high-fat diet compared to the DR and CON groups, but once put back on the chow pellet diet, they returned to CON level body weight gains, suggesting a transient effect of high-fat diet. Although DR and MIX groups were not different from each other in terms of body weight, the inclusion of the MIX group is unique to this study and proves advantageous in examining the effect of short term exposure to high-fat diet on cocaine's hypophagic and hyper-locomotor effects. Moreover, the MIX group presumably contains both DIO-prone and DR-prone rats, such that the group might be expected to show behavioral responses that are intermediate to those of DIO and DR rats. Indeed, after two weeks on the high-fat diet, the MIX group had weight gains that were intermediate of the DIO and DR group

The results of Experiment 1 replicated earlier findings, which noted that cocaine dose- dependently, reduced food intake and water intake while dose dependently increasing locomotion (Balapole et al., 1979; Berthold et al., 1992; Blavet and DeFeundis, 1982; Drouin et al., 2002a; Wellman et al., 2002; Wilson and Brenkert, 1978). Rats showed significant decreases in food intake after administration of 10 mg/kg cocaine and remained sensitive to cocaine's hypophagic and hypodipsic effects long after the first 60 min period after cocaine administration, showing depressed food intake into the second 60 min testing period (Panel B, Figure 3). Similar to food intake scores, water intake during the 60 min ingestive trial was suggestive of a dose dependent decrease. In contrast, a dose of 20 mg/kg of cocaine was required to induce a significant increase in locomotor activity that declined soon after the first hour. That is, generally, the hypophagic action of cocaine was evident at lower doses and persisted longer than did the locomotor effects of cocaine. Only the DR-prone group exhibited marked increase in locomotor scores after 10 mg/kg cocaine during the first hour after cocaine administration. This suggests that DR-prone rats are more reactive to the pharmacological action of cocaine prior to high-fat exposure and that DIO-prone rats are less reactive. Interestingly, when viewed in terms of reactivity to cocaine's hypophagic effects at 10 mg/kg (i.p.) as a function of body weight, future body weight gain predicted the extent to which food intake was reduced after administration of 10 mg/kg cocaine (as body weight gain increased, sensitivity to cocaine hypophagia decreased; see Panel A, Figure 11). Moreover, a similar plot of total distance traveled scores versus weight gain resembled the correlation seen in food intake data, suggesting that propensity for obesity confers a diminished response to cocaine, suggesting a common change in the function of a pathway common to both feeding and locomotion.

The factor of being resistant to obesity or prone to obesity appears to interact with the stimulant effects of cocaine and needs to be considered in future psychostimulant research. Inasmuch as other psychostimulants such as amphetamine and nicotine act to induce suppression of eating and hyperactivity, studies might be done to determine if the same relation holds for other psychostimulants such as amphetamine, sibutramine, and nicotine. In addition, as the results of Experiment 1 suggested, lower doses of cocaine (i.e. less than 10 mg/kg) should be used to investigate the subtle differences between the groups in terms of cocaine hypopagia. Until the neurochemical and physiological underpinnings of the interaction between the stimulant action of cocaine and the propensity for or resistance to obesity is mechanistically examined, the underlying basis for this effect is unknown.

The results of Experiment 2 suggested that exposure to a high-fat diet changed the cocaine sensitivity profile for the rats depending on the extent of weight gain. As expected, each group showed a dose dependent decrease in food intake and a dose dependent increase in locomotor activity in response to cocaine administration. Unlike Experiment 1, in Experiment 2, significant differences existed between the DIO group and the rest of the groups in terms of sensitivity to the hypophagic and hyper-locomotor effects of cocaine, with the DIO group generally eating less and locomoting more than the other groups after administration of 10 mg/kg cocaine. Thus, the most compelling finding in this study is that not only do rats' future body weight gain predict their reactivity to cocaine before and after exposure to high-fat diet, but that the predictions are in two different directions depending on whether cocaine reactivity is examined before or after the expression (or lack thereof) of obesity (see Fig. 11A and 11C).

There are a number of possible explanations for the enhanced reactivity to cocaine in DIO rats in Experiment 2. One explanation is that DIO rats were exposed to a series of cocaine injections in Experiment 1 and again in Experiment 2 and that the enhanced response in Experiment 2 reflects sensitization. It should be noted however that few injections were given in Experiment 1 (and in an ascending series) and that all groups except for the DIO group exhibited decreased cocaine hypophagia in the Experiment 2, suggesting that age and prior exposure to cocaine may affect DIO and DR rats differentially (compare CON group food intake in Experiment 1 (Fig. 3A) and Experiment 2 (Fig. 8A)). These differences argue against a simple change in tolerance and/or sensitization.

A second explanation is that the pharmacokinetics of cocaine are altered in obesity such that greater plasma and brain levels are achieved in obese rats relative to other rats. In other studies, increased carcass fat leads to increased reactivity to sibutramine, a 5-HT agonist (Strack et al., 2002). However, Bowman et al. (1999) suggests that there is no difference in the pharmacokinetics of cocaine between male and female rats, that differed in adipose fat stores when given cocaine, which argues against a a change in cocaine pharmacokinetics in obese rats.

A related argument is that the possibility that DIO rats are generally receiving more cocaine since total dosage is dependent on body weight. If this were the case, the rats in the present study should have shown hypo-sensitivity to cocaine hypophagia in Experiment 1 compared to Experiment 2 because all rats were significantly heavier in Experiment 2, but they did not. In addition, a comparison of food intake (g) and total distance scores (cm) of the heaviest and lightest rats for each experimental group indicated no significant difference.

Prior research indicates important genetic and physiological differences between between DIO-prone rats (before access to a high-energy diet) and DIO rats (after access to a to a high-energy diet) and between DIO rats and DR rats after exposure to a high-energy diet. These differences suggest a number of possible explanations for the increased reactivity of DIO rats to cocaine. In the following section, a few of these are explored although it is recognized that many possibilities exist in the explanation of the findings (Levin and Dunn-Meynell, 1997, 2000, 2002; Levin et al., 1997; Levin et al., 1986).

The hyper-sensitivity to cocaine administration after exposure to high-fat diet seen in DIO rats, and the hypo-sensitivity to cocaine in DR and MIX rats, confirms unpublished data collected in this lab. After chronic exposure to high-fat diet, DIO rats "normalize" and have a physiological profile similar to that of DR-prone rats, while DR rats do not normalize but show persistent increases in leptin as well as other adiposity signal levels (Levin and Dunn-Meynell, 2002). In a sense, there are three time points during which DIO and DR rats differ: 1) genetic predisposition prior to high-fat exposure (as discussed earlier) 2) physiological changes during rapid weight gain (or lack thereof) in response to high-fat diet and 3) shift in body weight set point to maintain current body weight. Thus, DR and DIO rats show two very different temporal profiles in response to high-fat exposure. Although both ends of the spectrum are covered in the present study (pre-high-fat exposure and after chronic exposure to high-fat diet), it is unknown how DIO and DR rats will react to cocaine stimulation after short term high-fat diet exposure.

Compelling data suggests a linkage between satiety signals and reward centers in brain. Injection of intracranial leptin reduces the reinforcing effects of brain stimulation (Fulton et al., 2000), while leptin, insulin, neuropeptide Y (NPY) and melanocortin (MC) receptors have been detected in reward centers of the brain (Figlewicz et al., 2003; Lindblom et al., 1999; Pickel et al., 1998). The reduced sensitivity of brain leptin receptors due to high plasma leptin levels in DIO rats may explain their hyper-sensitivity to cocaine reactivity observed in the present study (Levin and Dunn-Meynell, 2002). Since DIO rats showed reduced hypophagic effect as compared to DR rats when injected with leptin intracranially (Levin and Dunn-Meynell, 2002), leptin may not play a direct role in the enhancement of cocaine hypophagia, but it may be acting indirectly to enhance cocaine reinforcement. An indirect effect of leptin may be that leptin receptors exist on dopamine (DA) neurons whose cell bodies are located in the ventral tegmental area, and act to inhibit DA and NE release (Brunetti et al, 1999). The disinhibition of DA and NE neurons in the VTA and NA may act to increased basal neuron firing, and in turn, increase accumulation of DA and NE in the synapse when cocaine acts to inhibit monoamine transporters. The same logic can be applied to explain the hypo-sensitivity to cocaine seen in DR rats that do not show reduced sensitivity to plasma leptin levels.

Besides leptin, cocaine-amphetamine-regulated transcript (CART), an endogenous anorectic peptide (Kristensen, et al, 1998), may be another candidate for the differential sensitivity to cocaine, since CART has been shown to increase with administration of psychostimulants (Douglass et al., 1995), as well as induce hyperlocomotion in rats (Jaworski et al, 2003). CART mRNA levels increase in the hypothalamus with the increase of weight and short-term exposure to high-fat diet (Rohner-Jeanrenaud, et al, 2002), yet CART mRNA expression is low in leptin-deficient and leptin resistant rats that are obese without exposure to high-fat diet (Kristensen et al., 1998). This suggests that increased CART mRNA levels are a result of high-fat exposure resulting in obesity and not obesity per se. Since the present data suggested that high-fat exposure resulting in body weight gain above that of controls may play a role in increasing sensitivity to cocaine administration in the DIO group, it may be construed that the up-regulation of CART mRNA can predispose the system to over-express CART peptide in the presence of an agonist such as cocaine, and in turn increase suppression of food intake and increase hyperlocomotion. In support of this hypothesis, Pothos (2000) has suggested that the reason why rats maintained on a high-fat cafeteria diet have low basal levels of dopamine, yet release significantly higher levels of DA when given amphetamine (compared to chow-fed controls), is that obese rats release larger quantal sizes of DA when the system is activated. It must be noted that Pothos (2000) did not take propensity for obesity into consideration in his study.

In order to directly compare the results of the present study and existing data on lean and obese animals, the present data were analyzed based on high-fat exposure status. There was a slight increase in cocaine reactivity due to high-fat exposure (DIO and DR food intake data averaged and compared to MIX group and CON group). These results do not replicate the effects seen in studies that examine the effects of anorectic agents in rats maintained on high-fat diet. Clegg et al. (2003) have shown that increased dietary fat reduces the anorectic effects of intra-ventricular injection of melanocortin II (MTII), a melanocortin 3/4-receptor agonist, without change in mRNA expression of pro-opiomelanocortin (POMC), agouti-related peptide (AgRP) and melanocortin$_4$ receptors (MC4R), all of which are involved in the control of ingestive behavior. Loebens and Barros (2003) did in fact show no diet-dependent difference in cocaine-induced hyper-locomotor activity during a swim test, but exposure to high-fat diet was for only 22 days relative to the 11 weeks of high-fat exposure in the present study. A possible explanation for the discrepancy between the present results and pre-existing data is that the testing procedures in the present experiments relied upon a two week period where all groups were maintained on low-fat chow in order to equate results during testing, whereas other studies either tested animals using two different types of diets or using the same diet without prior maintenance of rats on low-fat chow (Pothos, 2000; Lin et al., 2000). It has been shown that palatable food has higher reward efficacy via increased dopamine release in the NAcc (Roop et al., 2002), and that food deprivation increases rewarding effects of drugs of abuse (Cabeza de Vaca and Carr, 1998). In addition, recent unpublished studies from this lab show that high-fat exposure results in greater anorexia and weight loss in response to nicotine administration than that noted in a chow control group. In addition, cholecystokinin (CCK) anorexia may vary as a function of the test diet employed (Torregrossa and Smith, 2003). In light of the data suggesting differential results due to test diet employed, pre-existing studies must contend with a factor that may complicate the interpretation of their results.

The concurrent quantification of food intake and locomotion has rarely been implemented in studies examining psychostimulant action. This is due in part to the fact that hypophagia is less well studied than are other aspects of psychostimulant action. Moreover, many studies of psychostimulant action take advantage of the observation that food deprivation enhances the reward properties of cocaine; thus providing food would be expected to diminish the reward properties of cocaine. In the present studies, the primary interest was the hypophagic action of cocaine so no food deprivation was used. However, rats did not have access to chow pellets one hour before testing. This was not considered to be food deprivation since the removal of food occurred before the start of the dark period when feeding was unlikely to occur and the duration was relatively short.

In summary, DR-prone rats exhibited greater reactivity to the locomotor stimulant properties of low dose of cocaine (10 mg/kg) as compared to the CON, MIX and DR groups, which suggests that a correlate of this syndrome may result in enhanced reactivity to the stimulant, but not the hypophagic effects of cocaine. In contrast, DIO rats were more reactive to both the locomotor and hypophagic actions of cocaine in Experiment 2.

Relatively little is known about the epidemiology of cocaine addiction in the obese population, since chronic high doses of cocaine induces weight loss in human subjects, precluding any analyses involving predisposition to obesity and cocaine sensitivity in the human population. Given that cocaine abuse in the human population often involves high doses rather than low doses of cocaine, an appropriate comparison in the present study are the cocaine effects that were observed when rats were administered doses of 20 and 30 mg/kg cocaine. At high doses, the groups did not exhibit differential sensitivity to cocaine, yet administration of a relatively low dose (10 mg/kg cocaine) revealed significant differences between the DIO group, and the CON, DR and MIX groups in terms of cocaine hypophagia and cocaine hyperlocomotion. This suggests that in order to fully investigate the role of genetic predisposition as well as expressed obesity in cocaine's hypophagic effects, doses lower than 10 mg/kg should be employed.

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